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(71) Applicant *(for all designated States except US)*: SCHERING AKTIENGESELLSCHAFT [DE/DE]; 13342 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BRINGMANN, Peter, W.** [DE/US]; 4468 Windflower Court, Concord, CA 94518 (US). **BRINK, Jody** [US/US]; 1630 Zinfandel Drive, Petaluma, CA 94954 (US). **HARKINS, Richard** [US/US]; 2819 Sea View Parkway, Alameda, CA 94502 (US). **LIGHT, David, R.** [US/US]; 614 South Fremont Street, San Mateo, CA 94402 (US). **LIN, Richard, J.** [US/US]; 1026 Hill Meadow Place, Danville, CA 94526 (US). **PARKES, Deborah** [US/US]; 19050 Hampton Place, Hayward, CA 94541 (US). **PARRY, Gordon** [GB/US]; 2255 Hackamore Court, Walnut Creek, CA 94596 (US). **SCHNEIDER, Douglas, W.** [US/US]; 3329 Walnut Lane, Lafayette, CA 94549 (US). **STEINBRECHER, Renate** [DE/US]; 2255 Hackamore Court, Walnut Creek, CA 94596 (US). **VAN HEUIT, Pamela, Toy** [US/US]; 3965 Campolindo Drive, Moraga, CA

[Continued on next page]

(54) Title: DNA ENCODING PROST 07 POLYPEPTIDE

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  1 MTAAGNPWGWFLGYLLLGITGSLAFINGGHIINGEDCNPHSQPWQAALFL 50
                          |||   .   |||||||.|||||||||||  :
  1 ....................GSLVSGSCSQIINGEDCSPHSQPWQAALVM 30

 51 EDDFFCGGVLVHPQWVLSAAHCFQNSYTIGLGLHNLEPEQEPGSQMMEAS 100
    |.:  || ||||||||||||||||||||||||||||.||  :||||||||.|||
 31 ENELFCSGVLVHPQWVLSAAHCFQNSYTIGLGLHSLEADQEPGSQMVEAS 80

101 LSIQHPEYNEPSMANDLMLIKLKESVSLSDTVRNISVVSQCPTPGDSCLV 150
    ||:.|||||  | :|||||||||| |||| |||:|.||: |||||  |.||||
 81 LSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLV 130

151 SGWGRLASGRLPQVLQCVNISVASEEVCKARYGPVYHPSMFCAGGGQDQK 200
    ||||  ||.||:|  |||||||:|| |||||    | |.|||||||||||||
131 SGWGLLANGRMPTVLQCVNVSVVSEEVCSKLYDPLYHPSMFCAGGGQDQK 180

201 DSCHGDSGGPLICNGSLQGLVSFGQAQCGQPNVPGVYTNLCKFTDWIQTT 250
    |||.|||||||||||  |||||||||.|  |||  |||||||||||||:||: |
181 DSCNGDSGGPLICNGYLQGLVSFGKAPCGQVGVPGVYTNLCKFTEWIEKT 230

251 IQAS* 255
    :||||
231 VQAS* 235
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(57) Abstract: The present invention relates to novel serine protease polypeptides, designated PROST 07, polynucleotides encoding the polypeptides, methods for producing the polypeptides, expression vectors and genetically engineered host cells for expression of the polypeptides. The invention further relates to methods for utilizing the polynucleotides and polypeptides in research, diagnosis, and therapeutic applications.

WO 01/25446 A1

94556 (US). **XUAN, Jian-Ai** [US/US]; 6729 Kit Lane, Castro Valley, CA 94591 (US).

(74) Agents: ZELANO, Anthony, J.; Millen, White, Zelano & Branigan, P.C., Arlington Courthouse Plaza I, Suite 1400, 2200 Clarendon Boulevard, Arlington, VA 22201 et al. (US).

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DNA ENCODING PROST 07 POLYPEPTIDE

This application claims the benefit of U. S. Provisional Application No. 60/158,588, filed October 7, 1999, which is incorporated herein in full by reference.

Field of the Invention

This invention relates, in part, to newly identified polynucleotides and polypeptides; variants and derivatives of the polynucleotides and polypeptides; methods of making the polynucleotides and polypeptides, and their variants and derivatives; antibodies directed toward the polypeptides, their variants and derivatives; and uses of the polynucleotides, polypeptides, variants, derivatives and antibodies. In particular, in these and in other regards, the invention relates to novel PROST 07 polypeptides, polynucleotides which encode these polypeptides, antibodies directed toward these polypeptides, and antisense polynucleotides that block PROST 07 expression.

BACKGROUND OF THE INVENTION

Prostate cancer is a frequently occurring disease in man, in that it is found in about one third of men over the age of 45. There is evidence for both genetic and environmental causes, with the majority of cases probably being the result of a combination of both factors. Studies of familial cancer have suggested that genetic predisposition plays a role in about 5-10% of all prostate cancers, and in about 45% of cases in men younger than 55.

There is evidence that prostate cancer develops as a multi-step disease, with one of the precursor lesions being prostatic intraepithelial neoplasia (PIN). Early stages of the disease are androgen dependent, while later stages are hormone independent. A proliferative disorder of the prostate known as benign prostatic hyperplasia is often detected clinically but is probably not a stage in the development of cancer. It is, however, frequently associated with prostate cancer. Cancers in the prostate are often multifocal, generally slow growing, and heterogeneous. Late stage cancers frequently metastasize to the lymph nodes and to the bone.

Prostate cancer is usually diagnosed by physical examination and by serum levels of prostate specific antigen (PSA). Radical prostatectomy is the treatment of choice for localized disease. Advanced metastatic disease is treated currently by androgen ablation induced by orchiectomy or treatment with GnRH (gonadotrophin releasing hormone), and by anti-androgen therapy. However, advanced disease almost invariably becomes hormone resistant and there is no cure for progressive disease. Moreover, there are serious side

effects associated with both radical prostatectomy and androgen ablation therapy. These include a high risk of incontinence and impotence associated with radical prostatectomy and bone fractures and osteoporosis associated with androgen ablation therapy.

There is, therefore, a considerable need for new therapeutic approaches for both early and late stage prostate cancer. There is also a significant need for new diagnostic agents, in particular agents that can discriminate stages of the disease, as this significantly influences the treatment options. For example, if disease has progressed beyond the prostate and has metastasized to the lymph nodes, radical prostatectomy is not undertaken as it has no effect on progression, but may have significant unwanted side effects. An agent that could detect metastasis, *in vivo*, would have considerable value.

Changes in the expression of specific proteins have been demonstrated in prostate cancer including abnormal p53 expression in late stage prostate cancer, reduced levels of TGF-β receptors, reduced levels of E-cadherin, C-CAM (a cell adhesion molecule), and several integrins. The expression of the oncogene bcl-2 is strikingly elevated in late stage androgen independent tumors, and prognosis for patients expressing bcl-2 at elevated levels is relatively poor. While the previously mentioned changes in gene expression are well documented, no changes in expression have been identified that have been demonstrated to be causative for the disease. It would, therefore, be useful to identify new proteins whose expression is linked to the presence or development of prostate tumors which could serve as molecular targets for prostate cancer diagnosis and therapy.

This invention discloses a new human serine protease that is a homologue to the human kallikrein gene family. This homologue, designated PROST 07, is differentially expressed in prostate tissue as compared with other tissues, and may be over-expressed in prostate tumors.

The kallikreins are a group of serine proteases that are involved in the postranslational processing of specific polypeptide precursors (Clements, *Endocr. Rev.* 10:393-419, 1989; Young et al., *J. Androl.* 16: 97-99, 1995). The human genome contains three kallikrein members: pancreatic/salivary/renal kallikrein (hK1), glandular kallikrein-2 (hK2), and prostate specific antigen (PSA or hK3) (Young et al., *J. Androl.* 16: 97-99, 1995; Carbini et al., *J. Hypertens.* 11:893-898, 1993). All three kallikrein genes are located as a cluster and span 80kb on chromosome 19q13.2-q13.4. (Riegman et al., *Genomics* 14: 6-11, 1992). The DNA (65-80%) and amino acid (57-78) sequence homologies of the three genes and protein products suggest that they evolved by gene duplication from the same ancestral gene (Schedlich et al., *DNA* 6:429-437, 1987; Morris, *Physiol.* 16: 345-351, 1989).

Recently, a novel serine protease with homology to kallikreins and trypsins was isolated and characterized from the developing enamel matrix in porcine teeth (Simmer et al., *J. Dent. Res.* 77: 377-387, 1998). This serine protease, named Enamel Matrix Serine Protease 1 (EMSP1) seems to be a porcine tooth specific protease and may play a key role in

both enamel and dentin formation. It has been shown that EMSP1 is activated by matrix metalloproteases in the outer enamel layer (Tanabe et al., *Arch. Oral Biol.* 39:277-281, 1996).

Two major types of serine proteases are involved in a wide range of proteolytic reactions. The first group, trypsin like enzymes, includes digestive (chymotrypsin, trypsin), lysosomal (cathepsin G) and fibrinolytic (plasminogen activators) enzymes. The second major group of serine proteases are homologous to the bacterial protease subtilisin. They act within the secretory pathway and cleave polypeptide precursors at specific basic sites, generating their biologically active forms. Serine protease inhibitors (serpins) constitute a family of proteins including at least α1-antitrypsin or α1-antichymotrypsin.

The contribution of serine proteases in tumor invasion and metastasis was initially thought to be the destruction of extracellular matrices. Recent evidence suggests, however, that serine proteases mainly affect tumor growth rather than invasion (Noel et al., *Invasion Metastasis* 17:221-239, 1997). Proteases have been shown to influence the release, activation and bioavailability of growth factors (Whitelock et al., *J. Biol. Chem.* 71: 10079-10086, 1996) and consequently modulate tumor cell growth, apoptosis and angiogenesis.

These data suggest that serine proteases are good candidates for use in the diagnosis of cancer and in possible therapeutic intervention. Based on the protein expression data of ourselves and others, we anticipate that the subject of the instant invention, the novel serine protease, PROST 07, will primarily find utility in the treatment and diagnosis of prostate, ovarian and endometrial cancers.

SUMMARY OF THE INVENTION

The present invention provides a polynucleotide sequence which uniquely encodes a novel serine protease designated herein as PROST 07. The PROST 07 polypeptide is characterized by a catalytic triad composed of serine, histidine and aspartic acid as has been described for other serine proteases. It shows homology to the kallikrein family of serine proteases and shows an 82.9% similarity to the newly described serine protease, EMSP1. The polynucleotide sequence, designated herein as *prost 07*, and described herein in Figure 1 (SEQ ID NO: 1), encodes the amino acid sequence for PROST 07, which is shown in Figure 2 (SEQ ID NO: 2).

Toward these ends, and others, it is an object of the present invention to provide polypeptides, *inter alia*, that have been identified as novel serine proteases, by homology between the amino acid sequence set out in Figure 2 (SEQ ID NO: 2) and known amino acid sequences of other serine protease polypeptides.

It is a further object of the invention, moreover, to provide polynucleotides that

encode such polypeptides, particularly polynucleotides that encode the polypeptide designated herein as PROST 07.

In accordance with this aspect of the invention there are provided isolated polynucleotides encoding PROST 07, including mRNAs, cDNAs, genomic DNAs and, in further embodiments of this aspect of the invention, biologically, diagnostically, clinically or therapeutically useful variants, analogs or derivatives thereof, or fragments thereof, including fragments of the variants, analogs and derivatives.

Among the particularly preferred embodiments of this aspect of the invention are naturally occurring allelic variants of polynucleotides that encode variants of the polypeptide designated herein as PROST 07.

In accordance with this aspect of the invention there are provided novel polypeptides of human origin referred to herein as PROST 07 as well as biologically, diagnostically or therapeutically useful fragments, variants and derivatives thereof, variants and derivatives of the fragments, and analogs of the foregoing.

Among the particularly preferred embodiments of this aspect of the invention are variants of PROST 07 encoded by naturally occurring allelic variants of the *prost 07* polynucleotide.

It is another object of the invention to provide a method of producing the aforementioned polypeptides, polypeptide fragments, variants and derivatives, fragments of the variants and derivatives, and analogs of the foregoing. In a preferred embodiment of this aspect of the invention there are provided methods of producing the aforementioned PROST 07 polypeptides comprising culturing host cells having expressibly incorporated therein an exogenously-derived PROST 07-encoding polynucleotide under conditions for expression of human PROST 07 in the host and then recovering the expressed polypeptide.

In accordance with another object of the invention there are provided products, compositions, processes and methods that utilize the aforementioned polypeptides and polynucleotides for *inter alia* research, biological, clinical and therapeutic purposes.

In accordance with certain preferred embodiments of this aspect of the invention, there are provided products, compositions and methods, *inter alia*, for assessing PROST 07 expression in cells by determining PROST 07 polypeptides or PROST 07-encoding mRNA; and assaying genetic variation and aberrations, such as defects, in *prost 07* genes.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided probes that hybridize to *prost 07* sequences.

It is a further object of the invention to provide antibodies which are highly selective for PROST 07 polypeptides, or fragments thereof, and which may be employed in a method for diagnosis and/or detection of PROST 07 expression, which may be associated various cancers; i.e. prostate and ovarian. In accordance with certain preferred embodiments of this aspect of the invention, antibodies are labeled in such a way as to produce a detectable

signal. Particularly preferred would be an antibody labeled with a radiolabel, an enzyme, a chromophore or a fluorescer.

In a further aspect of the invention there are provided antibodies which are conjugated to a therapeutic agent for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. Particularly preferred in this regard are therapeutic agents which are cytotoxic. In certain preferred embodiments in this regard is administration of such conjugated antibodies to a human patient for treatment of a disease state characterized by PROST 07 activity or expression such as prostate or ovarian cancer.

In a further aspect of the invention, peptides and anti-idiotypic antibodies are provided which can be used to stimulate an immune response.

In a further aspect of the invention there are provided ribozymes and polynucleotides complementary to *prost 07* polynucleotides (i.e. antisense polynucleotides) for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. Particularly preferred in this regard is administration of antisense molecules to a human patient for treatment of a disease state, such as prostate cancer or benign prostatic hyperplasia, which is alleviated by decreasing the level of PROST 07 activity.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Polynucleotide sequence of *prost 07* (SEQ ID NO: 1), which encodes the biologically or immunologically active form of PROST 07.

FIGURE 2: Deduced amino acid sequence of the active form of PROST 07 (SEQ ID NO: 2), with the catalytic triad underlined.

FIGURE 3: Amino acid alignment of PROST 07 with the full-length sequence of enamel matrix serine protease (EMSP1). The sequence of PROST 07 is on the bottom.

FIGURE 4: Expression of *prost 07* mRNA in human tissues and cell lines by northern blot analysis. RNA from human tissues, both tumor and normal, and human cell lines was

examined for hybridization of *prost 07* mRNA to a radiolabelled probe. An mRNA species of 1.6 Kb in size was detected in human prostate tissues, both tumor and normal, in the human prostate tumor cell line LNCaP and in tumor tissue derived from LNCaP cells. No expression could be detected in tissues other than prostate tissue.

FIGURE 5: Expression of *prost 07* mRNA in human tissues by Taqman based PCR analysis. RNA from human tissues, both tumor and normal, was isolated by standard techniques. Primers and probe to detect *prost 07* mRNA expression were designed using Perkin Elmer's Primer Express software and synthesized by Synthetic Genetics. *Prost 07* mRNA was detected in human prostate tissues, both tumor and normal, but could not be detected in other normal and tumor tissues.

FIGURE 6: Effect of specific reduction of *prost 07* mRNA expression on prostate tumor cell growth. Three antisense oligonucleotides targeted to *prost 07* sequences (see Example 8) were utilized to determine the effect of specific reduction of *prost 07* mRNA in the PROST 07-expressing human prostate cell line LNCaP. Nuclear area is proportional to cell number.

FIGURE 7: PROST 07 activity measurements. Figure 7A shows zymography of purified GST-PROST 07. A sample of the glutathion eluate was fractionated in an SDS gel containing 10 % gelatin under non-reducing condition. To re-nature the separated proteins SDS was sequestered using Triton X-100 and the gel was stained with Coomassie. The zymogram identifies two enzymatic activities exhibiting apparent molecular weights of the GST-fusion protein and PROST 07 resulting from auto-catalysis. Lanes 1 and 3, different preparations of GST-PROST 07; lane 5, molecular weight markers. No sample was loaded in lanes 2 and 4. Figure 7B shows enzymatic assay of GST-PROST 07 activity. PROST 07 protease activity was determined using an enzymatic assay that follows the rate of hydrolysis of a peptide substrate (see Example 4). Proteolytic activity is shown as the percent material hydrolyzed over time.

FIGURE 8: Immunohistochemical staining of PROST 07. Polyclonal and monoclonal antibodies specific to PROST 07 were used to examine PROST 07 expression in a variety of human tissues as described in Example 3. Figure 8A shows staining of normal and prostate tumor tissue. Figure 8B shows staining of bone marrow containing prostate tumor metastasis.

FIGURE 9: Neutralization of PROST 07 activity by monoclonal antibodies. Mouse monoclonal antibodies, specific for PROST 07, were examined for their ability to neutralize

PROST 07 activity (see Example 6), using the chromogenic assay described in Example 4. The rate of release of chromogenic substrate is followed over time. Three monoclonal antibodies, 5F4, 4B8, and 4A12, were found to significantly inhibit PROST 07 activity.

FIGURE 10: Inhibition of tumor migration/invasion by monoclonal antibodies. A mouse monoclonal antibody specific for PROST 07 (4B8) was used to block migration (Figure 10A) or invasion (Figure 10B) of PROST 07 positive CAOV-3 cells (see Example 7). Control cells were either untreated or treated with non-specific IgG mouse antibody.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used in the specification, examples and appended claims, unless specified to the contrary, the following terms have the meaning indicated.

"PROST 07" refers to the polypeptide having the amino acid sequence set out in Figure 2 (SEQ ID NO: 2); variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 2 (SEQ ID NO: 2) mean a polypeptide which retains essentially the same biological and/or immunological activity as the polypeptide of Figure 2 (SEQ ID NO: 2).

"prost 07" refers to the polynucleotide having the sequence set out in Figure 1 (SEQ ID NO: 1) and polynucleotides encoding polypeptides having the amino acid sequence of PROST 07 set out in Figure 2 (SEQ ID NO: 2); and to polynucleotides encoding PROST 07 variants, analogs, derivatives and fragments, and fragments of the variants, analogs and derivatives. *Prost 07* also refers to such polynucleotides composed of RNA as well as to polynucleotides which are the complement of polynucleotides which encode the polypeptide sequence set out in Figure 2 (SEQ ID NO: 2).

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single-and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more

typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide.

As used herein, the term "polynucleotide" includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritium-labelled bases, to name just two examples, are polynucleotides as the term is used herein.

It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia*.

"Polypeptides", as used herein, includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes such as glycosylation and other post-translational modifications, or by chemical modification techniques which are well known in the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. Among the known modifications which may be present in polypeptides of the present invention are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a polynucleotide or polynucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA

mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance, I. E. Creighton, *Proteins-Structure and Molecular Properties*, 2nd Ed., W.H. Freeman and Company New York, 1993. Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., in *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York, pp 1-12, 1983; Seifter et al., *Meth. Enzymol.* 182: 626-646, 1990 and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging,* Ann. N.Y. Acad. Sci. 663: 48-62, 1992.

It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslational events, including natural processing events and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by entirely synthetic methods, as well.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli*, prior to proteolytic processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to efficiently express mammalian proteins having native patterns of glycosylation, *inter alia*. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present to the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

"Polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence encoding a polypeptide of the present invention, particularly the PROST 07 polypeptide having the amino acid sequence set out in Figure 2 (SEQ ID NO: 2). The term encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by introns) together with additional regions.

"Biological activity" refers to the structural, regulatory or biochemical functions of naturally occurring PROST 07 polypeptide.

"Immunologic activity" refers to the capability of the natural, recombinant or synthetic PROST 07, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Oligonucleotide(s)" refers to relatively short polynucleotides. Often the term refers to single-stranded deoxyribonucleotides, but it can refer as well to single- or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others. Oligonucleotides, such as single-stranded DNA probe oligonucleotides, often are synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of other methods, including *in vitro* recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms. "Oligonucleotides" or "oligomers" or polynucleotide "fragment", "portion", or "segment" refers to a polynucleotide sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides.

"Naturally occurring PROST 07" refers to PROST 07 produced by human cells that have not been genetically engineered and specifically contemplates various PROST 07 forms arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation, acylation, and cleavage.

"Variant(s)" of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail.

(1) A polynucleotide that differs in polynucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the polynucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

As noted below, changes in the polynucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations

are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Also as noted below, changes in the polynucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such polynucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below.

(2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination. Recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations may also be introduced to modify the properties of the polypeptide, to change ligand-binding affinities, interchain affinities, or polypeptide degradation or turnover rate.

"Allelic variant" refers to an alternative form of the *prost 07* polynucleotide. Alleles result from a mutation, i.e., a change in the polynucleotide sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, or one or more times in a given sequence.

"Derivative" refers to polynucleotides or polypeptides derived from naturally occurring *prost 07* or PROST 07, respectively, by chemical modifications such as ubiquitination, labeling (e.g., with radionuclides, various enzymatic modifications), pegylation (derivatization with polyethylene glycol) or by insertion or substitution of amino acids such as ornithine (or substitution of the nucleotides which code for such as an amino acid), which do not normally occur in human proteins.

"Deletion" is defined as a change in either polynucleotide or amino acid sequences in which one or more polynucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition" is that change in a polynucleotide or amino acid sequence which has resulted in the addition of one or more polynucleotides or amino acid residues, respectively, as compared to the naturally occurring polynucleotide or amino acid sequence.

"Substitution" results from the replacement of one or more polynucleotides or amino acids by different polynucleotides or amino acids, respectively.

Preferably, amino acid substitutions are the result of replacing one amino acid with

another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine, i.e. conservative amino acid replacement. Insertions or deletions are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in the polypeptide using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

"Fragment" is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned PROST 07 polypeptides and variants or derivatives thereof.

A polypeptide "fragment", "portion", or "segment" is a stretch of amino acid residues of at least about 5 amino acids, often at least about 7 amino acids, typically at least about 9 to 13 amino acids, and in various embodiments, at least about 17 or more amino acids.

"Recombinant" or "recombinant DNA molecule" refers to a polynucleotide sequence which is not naturally occurring, or is made by the artificial combination of two otherwise separated segments of sequence. By "recombinantly produced" is meant artificial combination often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of polynucleotides, e.g., by genetic engineering techniques. Such manipulation is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together polynucleotide segments with desired functions to generate a single genetic entity comprising a desired combination of functions not found in the common natural forms. Restriction enzyme recognition sites, regulation sequences, control sequences, or other useful features may be incorporated by design. "Recombinant DNA molecules" include cloning and expression vectors. "Recombinant" may also refer to a polynucleotide which encodes a polypeptide and is prepared using recombinant DNA techniques.

"Isolated" means altered "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated", but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs.

Polynucleotides and polypeptides may occur in a composition, such as media formulations, solutions for introduction of polynucleotides or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides

within the meaning of that term as it is employed herein.

"Substantially pure" and "substantially homogenous" are used interchangeably and describe PROST 07 polypeptide, or fragments thereof, or a polynucleotide segment encoding same, where such polypeptide or polynucleotide is separated from components that naturally accompany it. A PROST 07 polypeptide or fragment thereof, or DNA segment encoding same is substantially free of naturally-associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell in which it naturally originates will be substantially free from its naturally-associated components. Similarly, a polynucleotide that is chemically synthesized or synthesized in a cellular system different from the cell in which it naturally originated will be substantially free from its naturally-associated components.

"Homologous", when used to describe a polynucleotide, indicates that two polynucleotides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least 70% of the nucleotides, usually from about 75% to 99%, and more preferably at least about 98 to 99% of the nucleotides.

"Polymerase chain reaction" or "PCR" refers to a procedure wherein specific pieces of DNA are amplified as described in U.S. Pat. No. 4,683,195, issued 28 July 1987. Generally, sequence information from the ends of the polypeptide fragment of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will point towards one another, and will be identical or similar in sequence to opposite strands of the template to be amplified. The 5 terminal nucleotides of the two primers will coincide with the ends of the amplified material. PCR can be used to amplify specific DNA sequences from total genomic DNA, cDNA transcribed from total cellular RNA, plasmid sequences, etc. (See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, 51: 263, 1987; Erlich, ed., *PCR Technology*, Stockton Press, NY, 1989).

"Stringency" typically occurs in a range from about T_m (melting temperature)-5°C (5° below the T_m of the probe) to about 20°C to 25°C below T_m. As will be understood by those of skill in the art, a stringent hybridization can be used to identify or detect identical polynucleotide sequences or to identify or detect similar or related polynucleotide sequences. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

"Hybridization" as used herein, shall include "any process by which a polynucleotide strand joins with a complementary strand through base pairing" (Coombs, J., *Dictionary of Biotechnology*, Stockton Press, New York, N.Y., 1994).

"Therapeutically effective dose" refers to that amount of polypeptide or its antibodies, antagonists, or inhibitors, including antisense molecules and ribozymes, which ameliorate the

symptoms or conditions of a disease state. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, ED_{50}/LD_{50}.

"Treating" or "treatment" as used herein covers the treatment of a disease-state in a human patient, which disease-state is associated with prostate tumor growth and includes disease states in which the patient is in need of decreased levels of PROST 07.

Detailed Description of the Invention

The present invention relates to novel PROST 07 polypeptides, *prost 07* polynucleotides, and antibodies directed toward PROST 07 polypeptides, among other things, as described in greater detail below. In particular, the invention relates to novel PROST 07 polypeptides and the polynucleotides encoding these PROST 07 polypeptides, and relates especially to PROST 07 having the amino acid sequence set out in Figure 2 (SEQ ID NO: 2) and *prost 07* having the polynucleotide sequence set out in Figure 1 (SEQ ID NO: 1). The present invention also encompasses PROST 07 variants. A preferred PROST 07 variant is one having at least 70% similarity (preferably at least 70% identity) to the polypeptide sequence shown in Figure 2 (SEQ ID NO: 2) and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide shown in Figure 2 (SEQ ID NO: 2) and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide sequence shown in Figure 2 (SEQ ID NO: 2) and also includes portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

The coding sequence for the predicted active form of the PROST 07 polypeptide begins 31 base pairs from the 5 end of the nucleotide sequence shown in Fig 1 (SEQ ID NO: 1). The first 30 base pairs code for a predicted pro-sequence and part of a signal sequence.

The present invention is based in part on the identification of a catalytic triad characteristic of serine proteases that is identifiable in the PROST 07 polypeptide sequence. The triad is composed of a histidine residue, an aspartic acid residue, and a serine residue (positions 51, 96, and 187, respectively, in Figure 2 (SEQ ID NO: 2)). The amino acids surrounding these residues (underlined in Figure 2) are frequently conserved in serine proteases. The invention is also based in part on the structural homology shown in Figure 3 between PROST 07 and EMPS 1, another member of the serine protease family. The amino acid sequence of PROST 07 is approximately 77.8% identical to EMSP 1.

The invention is also based in part on the expression profile of PROST 07, as demonstrated by its expression in prostate tissue libraries and overexpression of PROST 07 in prostate tumor libraries. This is also seen in analysis of mRNA expression in tissue samples from normal and tumor tissues by Northern blotting and by PCR based Taqman analysis. Both methods of analysis demonstrated that mRNA encoding PROST 07 is expressed in prostate tissues but not in other tumor tissue samples or other normal tissue samples. However, recent studies have demonstrated the presence of PROST 07 in tissue samples from late-stage ovarian cancer patients (Obiezu, et.al., *Clin. Biochem.* 33:234, 2000), indicating that this type of cancer may also prove to be treatable with PROST 07 based therapeutic agents.

The invention is also based in part on functional studies demonstrating the effect of a reduction in the level of mRNA encoding PROST 07 on prostate tumor cell growth in culture. This was demonstrated in the prostate tumor cell line, LNCaP, using antisense oligonucleotides to reduce *prost 07* mRNA expression. Three antisense oligonucleotide reagents were selected that reduced mRNA levels for *prost 07* when administered to cells in the presence of lipids. Administration of these antisense oligonucleotide reagents to LNCaP cells inhibited proliferation of the cells in a dose dependent manner. In the same dose range, a control oligonucleotide mixture composed of a set of random 23mer oligonucleotides did not affect proliferation of the cells. These experiments then, demonstrated that inhibition of PROST 07 expression impacted the growth of the tumor cell lines in which PROST 07 is normally expressed.

The invention is further based on studies demonstrating the ability of antibodies directed against PROST 07 to both neutralize the activity of the protein and to block tumor cell migration and invasion *in vitro*.

Polynucleotides

In accordance with one aspect of the present invention, there are provided isolated polynucleotides that encode the PROST 07 polypeptide having the deduced amino acid sequence of Figure 2 (SEQ ID NO: 2).

Using the information provided herein, such as the polynucleotide sequence set out in Figure 1 (SEQ ID NO: 1), a polynucleotide of the present invention encoding a PROST 07 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA from cells of human tissue as starting material. Illustrative of the invention, the polynucleotide sequence in Figure 1 (SEQ ID NO: 1) was found in cDNA clones obtained from human prostate tissues. *Prost 07* was identified as a gene expressed in the prostate by mining Incyte's LifeSeq gene expression database. The mRNA coding for PROST 07 was discovered using the "Transcript Imaging" feature of

LifeSeq and search parameters that permitted selection of novel genes expressed in Incyte's prostate tumor cell cDNA libraries at an abundance greater than 2 copies per library. This set of genes was evaluated by electronic Northern analysis to determine their distribution in other prostate tissue libraries and in all the other tissue libraries in the database. Clone 1651585 was identified in library PROSTUT12 of LIFESEQ Version 4.1SEP96 (Beta), and shown to cluster with other clones containing *prost 07* polynucleotides in a prostate specific fashion by Electronic Northern analysis. This prostate specific expression has been seen consistently in updated versions of the LifeSeq database. All clones coding for fragments of PROST 07 were assembled into a contiguous sequence using Genetics Computer Group (GCG, Wisconsin Package) gene assembly program and the sequence of the assembled mRNA evaluated. An open reading frame coding for a protein was identified and the predicted protein was shown to be homologous to the family of serine proteases. Additional clones coding for fragments of PROST 07 were acquired from Incyte, including Clone 3357511, which contained the most complete 5' sequence in the LifeSeq database.

With a view to obtaining additional sequence at the 5' end of the *prost 07* polynucleotide sequence, 5 RACE PCR procedures were utilized (using an amplification kit obtained from Clontech) to generate additional cDNA products coding for PROST 07. A sample of cDNA generated from prostate tissue RNA from Clontech Laboratories was used as a template for these syntheses. These PCR products were cloned, and selected clones sequenced. These sequences extended the 5 sequence further to cover the predicted full length coding sequence of the active form of PROST 07 as well as the putative pro- sequence and part of a putative signal sequence. Additional sequence information was obtained from a genomic clone coding for PROST 07, (P1 clone onc77), and this confirmed the sequence determined by RACE PCR procedures. Sequence covering the 3 region of the transcript was obtained by sequencing Incyte clone 181800, which included the complete 3 region of the mRNA.

Polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof, or by methods described herein. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide shown in Figure 1 (SEQ ID NO: 1). It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of Figure 2 (SEQ ID NO: 2).

Polynucleotides of the present invention which encode the polypeptide of Figure 2 (SEQ ID NO: 2) may include, but are not limited to, the coding sequence for the polypeptide

itself; the coding sequence for the polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro-protein sequence; the coding sequence of the polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to, introns and non-coding 5 and 3 sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing (for example, splicing and polyadenylation signals) or additional coding sequences which code for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pTrcHisB vector (Invitrogen, Carlsbad, CA) among others, many of which are commercially available. As described in Gentz et al. (*Proc. Natl. Acad. Sci., USA* 86: 821-824, 1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived from influenza hemaglutinin protein, which has been described by Wilson et al. (*Cell* 37: 767, 1984), for instance.

The polynucleotides may encode a polypeptide which is the polypeptide plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the polypeptide (when the active form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a polypeptide from precursor to final form, may facilitate polypeptide trafficking, may prolong or shorten polypeptide half-life or may facilitate manipulation of a polypeptide for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be processed away from the polypeptide by cellular enzymes.

A precursor polypeptide, having the final form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called propolypeptides.

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 2 (SEQ ID NO: 2). A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by polynucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more polynucleotides. The variants may be altered

in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of PROST 07 set out in Figure 2 (SEQ ID NO: 2); variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding PROST 07 variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of the PROST 07 polypeptide of Figure 2 (SEQ ID NO: 2) in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the PROST 07 polypeptide. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polynucleotides encoding polypeptides having the amino acid sequence of Figure 2 (SEQ ID NO: 2) without substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical to a polynucleotide encoding the PROST 07 polypeptide having the amino acid sequence set out in Figure 2 (SEQ ID NO: 2), and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical to a polynucleotide encoding the PROST 07 polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these, those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological activity as the polypeptide encoded by the polynucleotide sequence of Figure 1 (SEQ ID NO: 1).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probes for cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding PROST 07 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the *prost 07* gene. Such probes generally will comprise at

least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases.

For example, the coding region of the *prost 07* gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a polynucleotide of the present invention is then used to screen a library of human cDNA, genomic DNA or mRNA to determine to which members of the library the probe hybridizes.

In sum, a polynucleotide of the present invention may encode a polypeptide, a polypeptide plus a leader sequence (which may be referred to as a prepolypeptide), a precursor of a polypeptide having one or more prosequences which are not the leader sequences of a prepolypeptide, or a prepropolypeptide, which is a precursor to a propolypeptide, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active forms of the polypeptide.

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the polypeptide fragments, polynucleotides that hybridize to polynucleotides encoding polypeptide fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode polypeptide fragments. In these regards, preferred polynucleotides are those that correspond to preferred polypeptide fragments, as discussed below.

Polypeptides

The present invention further relates to a PROST 07 polypeptide which has the deduced amino acid sequence of Figure 2 (SEQ ID NO: 2).

The invention also relates to fragments, analogs and derivatives of these polypeptides. The terms fragment, derivative and analog when referring to the polypeptide of Figure 2 (SEQ ID NO: 2) means a polypeptide which retains essentially the same biological activity as such a polypeptide. Thus, an analog includes a propolypeptide which can be activated by cleavage of the propolypeptide portion to produce an active polypeptide of the invention.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. In certain preferred embodiments, it is a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 2 (SEQ ID NO: 2) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group,

or (iii) one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol) or (iv) one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of PROST 07 set out in Figure 2 (SEQ ID NO: 2), variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile, interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe and Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the PROST 07 polypeptide of Figure 2 (SEQ ID NO: 2) in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the PROST 07 polypeptide. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of Figure 2 (SEQ ID NO: 2) without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention also include the polypeptide of Figure 2 (SEQ ID NO: 2) as well as polypeptides which have at least 70% similarity (preferably at least 70% identity) to the polypeptide of Figure 2 (SEQ ID NO: 2) and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of Figure 2 (SEQ ID NO: 2) and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of Figure 2 (SEQ ID NO: 2) and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the

sequence of a second polypeptide.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptides by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides.

Fragments

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of PROST 07, most particularly fragments of the PROST 07 of Figure 2 (SEQ ID NO: 2), and fragments of variants and derivatives of the PROST 07 of Figure 2 (SEQ ID NO: 2).

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned PROST 07 polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing," i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a PROST 07 polypeptide of the present invention comprised within a precursor polypeptide designed for expression in a host and having heterologous pre- and propolypeptide regions fused to the amino terminus of the PROST 07 fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from PROST 07.

As representative examples of polypeptide fragments of the invention, there may be mentioned those which have from about 25 to about 145 amino acids.

In this context "about" includes the particularly recited range and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes. For instance, about 145 amino acids in this context means a polypeptide fragment of 25 plus or minus several, a few, 5, 4, 3, 2 or 1 amino acids to 145 plus or minus several a few, 5, 4, 3, 2 or 1 amino acid residues, i.e., ranges as broad as 25 minus several amino acids to 145 plus several amino acids to as narrow as 25 plus several amino acids to 145 minus several amino acids.

Highly preferred in this regard are the recited ranges plus or minus as many as 5 amino acids at either or at both extremes. Particularly highly preferred are the recited ranges plus or minus as many as 3 amino acids at either or at both the recited extremes. Especially particularly highly preferred are ranges plus or minus 1 amino acid at either or at both extremes or the recited ranges with no additions or deletions. Most highly preferred of all in

this regard are fragments from about 25 to about 145 amino acids.

Among especially preferred fragments of the invention are truncation mutants of PROST 07. Truncation mutants include PROST 07 of Figure 2 (SEQ ID NO: 2), or variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus of the sequence shown in Figure 2 (SEQ ID NO: 2), or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out above also are preferred embodiments of truncation fragments, which are especially preferred among fragments generally.

Especially preferred in this aspect of the invention are fragments characterized by biological and/or immunological attributes of PROST 07. Most preferred are fragments containing the predicted active domain of PROST 07, which encompasses amino acids 11 to 234, or a fragment containing the putative proprotein sequence of PROST 07, encompassing amino acids 4-10.

Certain preferred regions in these regards are set out in Figure 2 (SEQ ID NO: 2), and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figure 2 (SEQ ID NO: 2). As set out in Figure 2 (SEQ ID NO: 2), such preferred regions include the catalytic triad found in serine proteases, which encompasses amino acids 47-52, 96-99, and 181-192 of PROST 07.

Among highly preferred fragments in this regard are those that comprise regions of PROST 07 that combine several structural features, such as the features set out above. In this regard, the active domain defined by the amino acid residues from about 11-234 of Figure 2 (SEQ ID NO: 2), which is characteristic of the serine protease family of proteins, is an especially highly preferred region. Such regions may be comprised within a larger polypeptide or may be by themselves a preferred fragment of the present invention, as discussed above. It will be appreciated that the term "about" as used in this paragraph has the meaning set out above regarding fragments in general.

Further preferred regions are those that mediate activities of PROST 07. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of PROST 07, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are fragments that contain regions that are homologs in sequence, or in position, or in both sequence and position to active regions of related polypeptides, such as the other proteins of the serine protease family, which includes PROST 07.

Vectors, host cells, and expression systems

The present invention also relates to vectors which include polynucleotides of the

present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Such techniques are described in Sambrook et al., *Molecular Cloning, A Laboratory Manual,* Cold Spring Harbor Press, Plainview, N.Y., 1989 and Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y., 1989.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. For instance, polynucleotides may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus, for instance, polynucleotides of the invention may be transfected into host cells with another, separate, polynucleotide encoding a selectable marker, using standard techniques for cotransfection and selection in, for instance, mammalian cells. In this case, the polynucleotides generally will be stably incorporated into the host cell genome.

Alternatively, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation also may be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook et al. cited above, which is illustrative of the many laboratory manuals that detail these techniques. In accordance with this aspect of the invention, the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors, also may be and preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the

host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, *inter alia*, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and the like, previously used with the host cell selected for expression generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV4O, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements. such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard. A preferred vector is one derived from baculovirus.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill. Suitable procedures in this regard, and for constructing expression vectors using alternative techniques, which also are well known and routine to those of skill, are set forth in great detail in Sambrook et al. cited elsewhere herein.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV4O early and late promoters and promoters of

retroviral LTRs, to name just a few of the well-known promoters. It will be understood that numerous promoters not mentioned are suitable for use in this aspect of the invention, are well known and may readily be employed by those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline, theomycin, kanamycin or ampicillin resistance genes for culturing *E. coli* and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as *E. coli. Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells, preferably insect cells BTI-TN-5B1-4. Hosts for a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

Various mammalian cell culture systems can be employed for expression, as well. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblast (Gluzman et al., *Cell* 23: 175, 1991). Other cell lines capable of expressing a compatible vector include for example, the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines. In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the polynucleotide sequence coding for PROST 07 may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a

nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing PROST 07 in infected host cells (Logan and Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-59, 1984). In addition, transcription enhancers, such as the rouse sarcoma virus (RVS) enhancer, may be used to increase expression in mammalian host cells.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which such a sequence of the invention has been inserted. The sequence may be inserted in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE7O, pQE6O and pQE-9, available from Qiagen USA (Valencia, CA); pBS vectors, Phagescript® vectors, Bluescript® vectors, pNH8A, pNHl6a, pNHl8A, pNH46A, available from Stratagene (LaJolla, CA); and ptrc99a, pK223-3, pKK233-3, pDR54O, pRIT5 available from Pharmacia Biotech (Piscataway, N.J.). Most preferred is the pTrcHisB vector, available from Introgen. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, PXTl and pSG available from Stratagene; and PSVK3, pBPV, pMSG and pSVL available from Pharmacia Biotech. Most preferred is the vectors pCineo vector available from Promega. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("cat") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two such vectors are pKK232-B and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known bacterial promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli* lacI and lacZ promoters,

the T3 and T7 promoters, the T5 tac promoter, the lambda PR, PL promoters, the trp promoter, and the trc hybrid promoter, which is derived from the trp and lac promoters. Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV4O promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV") and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Polypeptides can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., cited elsewhere herein.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cisacting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV4O enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5 to a ribosome binding site. The ribosome binding site will be 5 to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiating AUG. Also, generally, there will be a translation stop codon at the end of the

polypeptide and there will be a polyadenylation signal and a transcription termination signal appropriately disposed at the 3' end of the transcribed region.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals. The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous fuctional regions. Thus for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, special regions also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. For example, when large quantities of PROST 07 are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the *prost 07* coding sequence may be ligated into the vector in frame with sequence for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heede and Shuster, *J. Biol. Chem.* 264:5503-5509, 1989) and the like. PtrcHis vectors (Invitrogen, Carlsbad, CA) may be used to express foreign polypeptides as fusion proteins containing a polyhistidine (6xHis) tag for rapid purification. Proteins made is such systems are designed to include cleavage sites, such as an enterokinase cleavage site, so that the cloned polypeptide of interest can be released from the fusion peptide moiety at will.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, where the selected promoter is inducible it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period.

Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

The PROST 07 polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite

chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification. Various other methods of protein purification well known in the art include those described in Deutscher, M., *Methods in Enzymology*, Vol 182, Academic Press, San Diego, 1982; and Scopes, R., *Protein Purification: Principles and Practice* Springer-Verlag, New York, 1982.

Alternatively, the polypeptides of the present invention can be produced by direct peptide synthesis using solid-phase techniques (Stewart et al., *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, 1969; Merrifield, J., *J. Am. Chem. Soc.* 85:2149-2154, 1963). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif) in accordance with the instructions provided by the manufacturer. Various fragments of PROST 07 may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Uses of PROST 07 polypeptides and the polynucleotides which encode them

Prost 07 polynucleotides and PROST 07 polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of PROST 07. Additional applications relate to diagnosis and to treatment of diseases of cell proliferation, such as prostate cancer. These aspects of the invention are illustrated further by the following discussion and are described further within the body of the specification.

The rationale for the use of the polynucleotide and polypeptide sequences of the present invention is based in part on the chemical and structural homology between the PROST 07 disclosed herein and other serine protease molecules and on the preferential expression of PROST 07 in prostate tissues as compared with other tissues. PROST 07 may be used in the diagnosis and treatment of conditions, disorders or diseases associated with inappropriate growth of prostate tissue. These would include, but are not limited to, cancer and metastatic tumor growth.

Prost 07 polynucleotide sequences can be used as DNA probes, and as targets for antisense and ribozyme therapy, or as templates for the production of antisense polynucleotides.

PROST 07 polypeptides can be used to generate antibodies to PROST 07 which may be useful in detecting the levels of PROST 07 polypeptide in cells and tissues and in targeting drugs to primary and metastatic tumors.

PROST 07 polypeptides may be used to stimulate an immune response to PROST 07 containing cells.

Polynucleotides encoding PROST 07 may be useful in diagnostic assays for detecting the levels of polynucleotides encoding PROST 07 in cells and tissues.

In conditions associated with expression of PROST 07, such as prostate cancer, it may be advantageous to suppress expression or activity of PROST 07. PROST 07 expression could be suppressed by administration of antisense oligonucleotides or ribozymes. Alternatively, antibodies specifically recognizing areas of the PROST 07 polypeptide which are responsible for its activity may be administered to treat diseases or conditions associated with PROST 07 activity.

Polynucleotide assays

This invention is also related to the use of the *prost 07*-related polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of *prost 07* polynucleotides associated with a disease state will provide a tool for the development of *in vitro* and *in vivo* diagnostics that can add or define a diagnosis of a disease or susceptibility to a disease which results from tissue specific expression of PROST 07.

Individuals carrying mutations in the gene encoding PROST 07 may be detected at the DNA level by a variety of techniques. Polynucleotide samples for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis (Saiki et al., *Nature*, 324: 163-166, 1986). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the polynucleotide sequence encoding PROST 07 can be used to identify and analyze *prost 07* expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled *prost 07* RNA or alternatively, radiolabeled *prost 07* antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also

may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled polynucleotide or by automatic sequencing procedures with fluorescent tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see e.g., Myers et al., *Science*, 230: 1242, 1985).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Catton et al., *Proc. Natl. Acad. Sci., USA*, 85:4397-4401, 1985).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA).

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

Polypeptide assays

The present invention also relates to a diagnostic assays such as quantitative and diagnostic assays for detecting levels of PROST 07 polypeptide in cells and tissues and body fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of PROST 07 polypeptide compared to normal control tissue samples may be used to detect the presence of neoplasia, for example, prostate cancer. Such diagnostic tests may be used to detect metastatic tumor growth, as well. Assay techniques that can be used to determine levels of a polypeptide, such as a PROST 07 polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays (RIA), competitive-binding assays, western Blot analysis and enzyme-linked immunoabsorbant assays (ELISA), and fluorescent activated cell sorting (FACS). Among these ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to PROST 07, preferably a monoclonal antibody. In addition a reporter

antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as a radioactive, fluorescent or enzymatic reagent, in this example horseradish peroxidase enzyme.

To carry out an ELISA a sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the polypeptides in the sample. Any free polypeptide binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any PROST 07 polypeptides attached to the polystyrene dish. Unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to PROST 07. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to PROST 07 through the primary and secondary antibodies, produces a colored reaction product. The amount of color developed in a given time period indicates the amount of PROST 07 polypeptide present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to PROST 07 are attached to a solid support and labeled PROST 07 and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of PROST 07 in the sample.

These and other assays are described, among other places, in Hampton et al. (*Serological Methods, a Laboratory Manual*, APS Press, St Paul, Minn, 1990) and Maddox et al. (*J. Exp. Med.* 158:12111, 1983).

Antibodies

The invention further relates to antibodies that specifically bind to PROST 07, herein referred to as PROST 07 antibodies. The prostate-specificity of PROST 07 and its cell surface location represent characteristics of an excellent marker for screening, diagnosis, prognosis, follow-up assays and imaging methods. In addition, these characteristics indicate that PROST 07 may be an excellent target for therapeutic methods such as targeted antibody therapy, immunotherapy, and gene therapy. As used herein, the term "specifically binds to" refers to the interaction of an antibody and a polypeptide, in which the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the polypeptide; in other words, the antibody is recognizing and binding to a specific polypeptide structure rather than to proteins in general.

The PROST 07 polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto

(Harlow, *Antibodies*, Cold Spring Harbor Press, NY (1989)). These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and human antibodies, as well as Fab fragments, or the product of a Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, *Nature* 256: 495-497, 1975), the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* 4: 72, I983) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., in *Monoclonal Antibodies and Cancer*, Alan R. Liss, Inc., 77-96, 1985).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855, 1984; Neuberger et al., *Nature* 312:604-608, 1984; Takeda et al., *Nature* 314:452-454, 1985). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce PROST 07-specific single chain antibodies.

Furthermore , "human" antibodies can be produced using the methods described in U.S. Patent Nos. 5,877,397 and 5,569,825, which are incorporated herein in full by reference.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al. (*Proc. Natl. Acad. Sci. USA* 86:3833-3837, 1989) and Winter and Milstein (*Nature* 349:293-299, 1991).

Antibody fragments which contain specific binding sites for PROST 07 may also be generated. For example, such fragments include, but are not limited to the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al., *Science* 256:1270-1281, 1989).

The amino acid sequence of PROST 07 presented herein may be used to select specific regions of the PROST 07 polypeptide for generating antibodies. As will be understood by those skilled in the art, the regions or epitopes of a PROST 07 polypeptide to which an antibody is directed may vary with the intended application. For example, antibodies intended for use in an immunoassay for the detection of membrane-bound PROST 07 on prostate cells should be directed toward accessible epitopes on the PROST 07 polypeptide. Regions of the PROST 07 polypeptide that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating anti-PROST 07 antibodies. Particularly useful fragments include, but are not limited to, the sequences SQPWQAALMENELF (SEQ ID NO: 10), EASLSVRHPEYNRP (SEQ ID NO: 11), SKLYDPLYHPSMF (SEQ ID NO: 12), and CKFTEWIEKTVQAS (SEQ ID NO: 13). Generation of polyclonal antibodies to these regions is described in Example 5.

PROST 07 antibodies of the invention may be particularly useful in diagnostic assays, imaging methodologies, and therapeutic methods for the management of prostate cancer. The invention provides various immunological assays useful for the detection of PROST 07 polypeptides and for the diagnosis of prostate cancer. Such assays generally comprise one or more PROST 07 antibodies capable of recognizing and binding a PROST 07 polypeptide. The most preferred antibodies will selectively bind to PROST 07 and will not bind (or bind weakly) to non-PROST 07 polypeptides. The assays include various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunoabsorbent assays, and the like. In addition, immunological imaging methods capable of detecting prostate cancer are also provided by the invention, including but not limited to radioscintigraphic imaging methods using labeled PROST 07 antibodies. Such assays may be clinically useful in the detection, monitoring and prognosis of prostate cancer.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Additionally, PROST 07 antibodies may be used to isolate PROST 07 positive cells using cell sorting and purification techniques. In particular, PROST 07 antibodies may be used to isolate prostate cancer cells from xenograft tumor tissue, from cells in culture, etc. using antibody-based cell sorting or affinity purification techniques. Other uses of the PROST 07 antibodies of the invention include generating anti-idiotypic antibodies that mimic the PROST 07 polypeptide.

The PROST 07 antibodies can be used for detecting the presence of prostate cancer or tumor metastasis. The presence of such PROST 07-containing cells within various

biological samples, including serum, prostate and other tissue biopsy specimens, may be detected with PROST 07 antibodies. In addition, PROST 07 antibodies may be used in various imaging methodologies such as immunoscintigraphy with Tc-99m (or other isotope) conjugated antibody. For example, an imaging protocol similar to the one recently described using an In-111 conjugated anti-PSMA antibody may be used to detect recurrent and metastatic prostate carcinomas (Sodee et al., *Clin. Nuc. Med.* 21: 759-766, 1997).

The PROST 07 antibodies of the invention may be labeled with a detectable marker or conjugated to a second molecule, such as a cytotoxic agent, and used for targeting the second molecule to a PROST 07 positive cell (Vitetta, E.S. et al., *Immunotoxin Therapy*, in DeVita, Jr, V.T. et al., eds, Cancer: Principles and Practice of Oncology, 4th ed., J.B. Lippincott Co., Philadelphia, 2624-2636, 1993). Examples of cytotoxic agents include, but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diptheria toxin, Pseudomonas exotoxin(PE) A, PE40, abrin, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Suitable radioisotopes include the following: Antimony-124, Antimony-125, Arsenic-74, Barium-103, Barium-140, Berylllium-7, Bismuth-j206, Bismuth-207, Cadmium-109, Cadmium-115m, Calcium-45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Cobalt-56, Cobalt-57, Cobalt-58, Cobalt-60, Cobalt-64, Erbium-169, Europium-152, Gadolinium-153, Gold-195, Gold-199, Hafnium-175, Hafnium-175-181, Indium-11, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-210, Manganese-54, Mercury-197, Mercury-203, Molybdenum-99, Neodymium-147, Neptunium-237, Nickel-63, Niobiumo-95, Osmium-185+191, Palladium-103, Platinum-195m, Praseodymium-143, Promethium-147, Protactinium-233, Radium-2226, Rhenium-186, Rubidium-86, Ruthenium-103, Ruthenium-106, Scandium-44, Scandium-46, Selenium-75, Silver-110m, Silver-11, Sodium-22, Strontium-85, Strontium-89, Strontium-90, Sulfur-35, Tantalum-182, Technetium-99m, Tellurium-125, Tellurium-132, Thallium-204, Thorium-228, Throium-232, Thallium-170, Tin-113, Titanium-44, Tungsten-185, Vanadium-48, Vanadium-49, Ytterbium-169, Yttrium-88, Yttrium-90, Yttrium-91, Zinc-65, and Zirconium-95.

Immunotherapy for Prostate Cancer

The invention provides various immunotherapeutic methods for treating prostate cancer, including antibody therapy, *in vivo* vaccines, and *ex vivo* immunotherapy approaches. In one approach, the invention provides PROST 07 antibodies which may be used systemically to treat prostate cancer. For example, unconjugated PROST 07 antibodies may be introduced into a patient such that the antibody binds to PROST 07 on or in prostate cancer cells and mediates the destruction of the cells, and the tumor, by mechanisms which

may include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity, altering the physiologic function of PROST 07, and/or the inhibition of ligand binding or signal transduction pathways. PROST 07 antibodies conjugated to toxic agents such as ricin or radioisotopes may also be used therapeutically to deliver the toxic agent directly to PROST 07-bearing prostate tumor cells and thereby destroy the tumor cells.

Prostate cancer immunotherapy using PROST 07 antibodies may follow the teachings generated from various approaches which have been successfully employed with respect to other types of cancer, including but not limited to colon cancer (Arlen et al., *Crit. Rev. Immunol.* 18: 133-138, 1998), multiple myeloma (Ozaki et al., *Blood* 90: 3179-3186, 1997; Tsunenari et al., *Blood* 90: 2437-2444, 1997), gastric cancer (Kasprzyk et al, *Cancer Res.* 52: 2771-2776, 1992), B-cell lymphoma (Funakoshi et al., *Immunther. Emphasis Tumor Immunol.* 19: 93-101, 1996), leukemia (Zhong et al., *Leuk. Res.* 20: 581-589, 1996), colorectal cancer (Moun et al., *Cancer Res.* 54: 6160-6166, 1994; Velders et al., *Cancer Res.* 55:4398-4403, 1995), and breast cancer (Shepard et al., *J. Clin. Immunol.* 11: 117-127, 1991).

The invention further provides vaccines formulated to contain a PROST 07 polypeptide or fragment thereof. The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., *Int. J. Cancer* 63: 231-237, 1995; Fong et al., *J. Immunol.* 159: 3113-3117, 1997). Such methods can be readily practiced by employing a PROST 07 polypeptide, or fragment thereof, or a PROST 07-encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the PROST 07 immunogen.

For example, viral gene delivery systems may be used to deliver a PROST 07-encoding nucleic acid molecule. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, in *Curr. Opin, Immunol.* 8: 658-663, 1996). Non-viral delivery systems may also be employed by using naked DNA encoding a PROST 07 polypeptide or fragment thereof introduced into the patient (i.e., intramuscularly) to induce an anti-tumor response. In one embodiment, the full-length human *prost 07* cDNA may be employed. In another embodiment, human *prost 07* cDNA fragments may be employed. In another embodiment, *prost 07* nucleic acid molecules encoding specific T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific algorithims (e.g., Epimer, Brown University) to identify peptides within a PROST 07 polypeptide which are capable of optimally binding to specified HLA alleles.

Various *ex vivo* strategies may also be employed. One approach involves the use of dendritic cells to present a PROST 07 polypeptide as antigen to a patient's immune system.

Dendritic cells express MHC class I and II, B7 costimulator, and IL-12, and are thus highly specialized antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed with peptides of the prostate-specific membrane antigen (PSMA) are being used in a Phase I clinical trial to stimulate prostate cancer patients' immune systems (Tjoa et al., *Prostate* 28: 65-69, 1996; Murphy et al., *Prostate* 29: 371-380, 1996). Dendritic cells can be used to present PROST 07 polypeptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with PROST 07 polypeptides capable of binding to MHC molecules. In another embodiment, dendritic cells are pulsed with the complete PROST 07 polypeptide. Yet another embodiment involves engineering the overexpression of the *prost 07* gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al., *Cancer Gene Ther.* 4: 17-25, 1997), retrovirus (Henderson et al., *Cancer Res.* 56: 3763-3770, 1996), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., *Cancer Res.* 57: 2865-2869, 1997), and tumor-derived RNA transfection (Ashley et al., *J. Exp. Med.* 186: 1177-1182, 1997).

Anti-idiotypic anti-PROST 07 antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a PROST 07 polypeptide. Specifically, the generation of anti-idiotypic antibodies is well known in the art and can be readily adapted to generate anti-idiotypic anti-PROST 07 antibodies that mimic an epitope on a PROST 07 polypeptide (see, for example, Wagner et al., *Hybridoma* 16: 33-40, 1997: Foon et al., *J. Clin. Invest.* 96: 334-342, 1995; Herlyn et al., *Cancer Immunol Immunother* 43: 65-76, 1996). Such an anti-idiotypic antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against tumor antigens.

Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing PROST 07. Using the PROST 07-encoding DNA molecules described herein, constructs comprising DNA encoding a PROST 07 polypeptide/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take up the construct and express the encoded PROST 07 polypeptide/immunogen. The PROST 07 polypeptide/immunogen may be expressed as a cell surface polypeptide or be secreted. Expression of the PROST 07 polypeptide/immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for a review, see information and references published at internet address www.genweb.com).

Anti-sense oligonucleotides, antisense vectors, and ribozymes

Anti-sense polynucleotides complementary to *prost 07* may be prepared synthetically. Such oligonucleotides may be delivered into cells with or without lipids that may assist uptake

of the anti-sense oligonucleotides into cells.

Alternatively, expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be also be used for construction and delivery of recombinant vectors which will express anti-sense *prost 07*. See, for example, the techniques described in Sambrook et al. (*supra*) and Ausubel et al. (*supra*).

The polynucleotides comprising the full length cDNA sequence and/or its regulatory elements enable researchers to use *prost 07* polynucleotides as an investigative tool in sense strands (Youssoufian and Lodish, *Mol. Cell. Biol*. 13:98-104, 1993) or antisense strands (Eguchi, et al., *Annu. Rev. Biochem.* 60:631-652, 1991) for the regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers, or larger fragments, can be designed from various locations along the coding or control regions.

Genes encoding PROST 07 can be turned off by transfecting a cell or tissue with expression vectors which express high levels of a desired *prost 07* polynucleotide fragment. Such constructs can flood cells with untranslatable sense or antisense sequences. Particularly preferred antisense sequences include, but are not limited to, CAACGGGUACUUGCAGGGCCUUG (SEQ ID NO: 17), ACCUCUGCAAAUUCACUGAGUGG (SEQ ID NO: 18), and UACUUGCAGGGCCUUGUGUCUUU (SEQ ID NO:19). Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modification of gene expression can be obtained by designing antisense molecules, DNA or RNA, to control regions of *prost 07*, ie, the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, eg, between -10 and +10 regions of the leader sequence, are preferred. The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA were reviewed by Gee, J.E. et al. (In Huber and Car, *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, N.Y., 1994).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA (U.S. Patent No. 4,987,071; WO 93/23057). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of RNA encoding PROST 07. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme

cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays (Irie et al., *Advance. Pharmacol.* 40:207-257, 1997).

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription or by DNA sequences encoding PROST 07. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecules or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. Increased stability can also be achieved by the inclusion of nontraditional bases such as inosine and queosine as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Methods for introducing antisense vectors into cells or tissues include those methods discussed infra and which are equally suitable for *in vivo*, *in vitro* and *ex vivo* therapy. For *ex vivo* therapy, antisense vectors are introduced into cells taken from the patient and clonally propagated for autologous transplant back into that same patient as presented in U.S. Pat. Nos. 5,399,493 and 5,437,994, disclosed herein by reference. Delivery by transfection and by liposome or other lipid based or non-lipid based agents are well known in the art.

Assays for Identifying Agents Binding to PROST 07

The present invention also relates to assays and methods which can be used to identify agents that bind to PROST 07. Specifically, agents that bind to PROST 07 can be identified by the ability of the PROST 07 ligand or other agent or constituent to bind to PROST 07 and/or the ability to inhibit/stimulate PROST 07 activity. Assays for PROST 07 activity (e.g. binding) using a PROST 07 polypeptide are suitable for use in high through-put screening methods. In one embodiment, the assay comprises mixing PROST 07 with a test agent or cellular extract. After mixing under conditions that allow association of PROST 07 with the agent or component of the extract, the mixture is analyzed to determine if the agent/component is bound to PROST 07. Binding agents/components are identified as being

able to bind to PROST 07. Alternatively or consecutively, PROST 07 activity can be directly assessed as a means for identifying agonists and antagonists of PROST 07 activity, using a chromogenic assay such as that described in Example 4.

Alternatively, agents that bind to a PROST 07 polypeptide can be identified using a yeast two-hybrid system or a binding capture assay. In the yeast two hybrid system, an expression unit encoding a fusion protein made up of one subunit of a two subunit transcription factor and the PROST 07 polypeptide is introduced and expressed in a yeast cell. The cell is further modified to contain (1) an expression unit encoding a detectable marker whose expression requires the two subunit transcription factor for expression and (2) an expression unit that encodes a fusion protein made up of the second subunit of the transcription factor and a cloned segment of DNA. If the cloned segment of DNA encodes a protein that binds to the PROST 07 polypeptide, the expression results in the interaction of PROST 07 and the encoded protein. This brings the two subunits of the transcription factor into binding proximity, allowing reconstitution of the transcription factor. This results in expression of the detectable marker. The yeast two hybrid system is particularly useful in screening a library of cDNA encoding segments for cellular binding partners of PROST 07.

PROST 07 polypeptides which may be used in the above assays include, but are not limited to, an isolated PROST 07 polypeptide, a fragment of a PROST 07 polypeptide, a cell that has been altered to express a PROST 07 polypeptide, or a fraction of a cell that has been altered to express a PROST 07 polypeptide. Further, the PROST 07 polypeptide can be the entire polypeptide or a defined fragment of the PROST 07 polypeptide. It will be apparent to one of ordinary skill in the art that so long as the PROST 07 polypeptide can be assayed for agent binding, e.g. by a shift in molecular weight or activity, the present assay can be used.

The method used to identify whether an agent/cellular component binds to a PROST 07 polypeptide will be based primarily on the nature of the PROST 07 polypeptide used. For example, a gel retardation assay can be used to determine whether an agent binds to PROST 07 or a fragment thereof. Alternatively, immunodetection and biochip technologies can be adopted for use with the PROST 07 polypeptide. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent bind to a PROST 07 polypeptide.

Agents and cellular components can be further tested for the ability to modulate the activity of a PROST 07 polypeptide using a cell-free assay system or a cellular assay system. As the activities of the PROST 07 polypeptide become more defined, functional assays based on the identified activity can be employed. One such assay would be the assay of serine protease activity described in Example 4, using synthetic peptide substrates having a known serine protease cleavage site within their sequence.

As used herein, an agent is said to antagonize PROST 07 activity when the agent reduces PROST 07 activity. The preferred antagonist will selectively antagonize PROST 07,

not affecting any other cellular proteins. Further, the preferred antagonist will reduce PROST 07 activity by more than 50%, more preferably by more than 90%, most preferably eliminating all PROST 07 activity.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the PROST 07 polypeptide. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library, or growth broth of an organism or plant extract.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site an/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the PROST 07 polypeptide. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to a fragment of a PROST 07 polypeptide.

The agents tested in the methods of the present invention can be, as examples, peptides, antibodies, oligonucleotides, small molecules and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents used in the present screening method. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the PROST 07 polypeptide.

Peptide agents can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if no-gene-encoded amino acids are to be included.

Another class of agent of the present invention are antibodies immunoreactive with critical positions of the PROST 07 polypeptide. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the PROST 07 polypeptide intended to be targeted by the antibodies. Such agents can be used in competitive binding studies to identify second generation inhibitory agents as well as to block PROST 07 activity.

The cellular extracts tested in the methods of the present invention can be, as examples, agueous extracts of cells or tissues, organic extracts of cells or tissues or partially purified cellular fractions. A skilled artisan can readily recognize that there is no limit as to the source of the cellular extract used in the screening method of the present invention.

Agents that bind a PROST 07 polypeptide, such as a PROST 07 antibody, can be used to modulate the activity of PROST 07, to target anticancer agents to appropriate

mammalian cells, or to identify agents that block the interaction with PROST 07. Cells expressing PROST 07 can be targeted or identified by using an agent that binds to PROST 07.

How the PROST 07 binding agents will be used depends on the nature of the PROST 07 binding agent. For example, a PROST 07 binding agent can be used to: deliver conjugated toxins, such as diphtheria toxin, cholera toxin, ricin or pseudomonas exotoxin, to a PROST 07 expressing cell; modulate PROST 07 activity; to directly kill a PROST 07 expressing cell; or in screens to identify competitive binding agents. For example, a PROST 07 inhibitory agent can be used to directly inhibit the growth of PROST 07 expressing cells whereas a PROST 07 binding agent can be used as a diagnostic agent.

Pharmaceutical Compositions and Administration

The present invention also relates to pharmaceutical compositions which may comprise *prost 07* polynucleotides, PROST 07 polypeptides, antibodies, agonists, antagonists, or inhibitors, alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. Any of these molecules can be administered to a patient alone, or in combination with other agents, drugs or hormones, in pharmaceutical compositions where it is mixed with excipient(s) or pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert.

The present invention also relates to the administration of pharmaceutical compositions. Such administration is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tumor), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxilliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of *Remington's Pharmaceutical Sciences* (Ed. Maack Publishing Co, Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the

mixture of granules, after adding suitable auxilliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potatoe, or other plants; cellulose such as methyl, cellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, ie. dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations for parenteral administration include aqueous solutions of active compounds. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Kits

The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the

product for human administration.

Manufacture and Storage.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with may acids, including by not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

After pharmaceutical compositions comprising a compound of the invention formulated in an acceptable carrier have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of PROST 07, such labeling would include amount, frequency and method of administration.

Therapeutically Effective Dose.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose, i.e. treatment of a particular disease state characterized by PROST 07 expression. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, ED_{50}/LD_{50}. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations what include the ED_{50} with little or no toxicity. The dosage varies

within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, eg, tumor size and location; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature. See U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art will employ different formulations for polynucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

Example 1: Identification of human *prost 07* polynucleotide

Prost 07 was identified as a gene expressed in the prostate by mining Incyte's LifeSeq gene expression database. The mRNA coding for PROST 07 polypeptide was discovered using the "Transcript Imaging" feature of LifeSeq and search parameters that permitted selection of novel genes expressed in Incyte's prostate tumor cell cDNA libraries at an abundance greater than 2 copies per library. This set of genes was evaluated by electronic Northern analysis to determine their distribution in other prostate tissue libraries and in all the other tissue libraries in the database. Clone 1651585 was identified in library PROSTUT12 of LIFESEQ Version 4.1SEP96 (Beta) and shown to cluster with other clones containing *prost 07* polynucleotides in a prostate specific fashion by Electronic Northern

analysis. This prostate specific expression has been seen consistently in updated versions of the LifeSeq database. All clones coding for fragments of PROST 07 were assembled into a contiguous sequence using the Genetics Computer Group (GCG, Wisconsin package) gene assembly program and the sequence of the assembled mRNA evaluated. An open reading frame coding for a protein was identified and the predicted protein was shown to be homologous to the family of serine proteases. Additional clones coding for fragments of PROST 07 were acquired from Incyte, including Clone 3357511 that contained the most complete 5' sequence in the LifeSeq database.

With a view to obtaining additional sequence at the 5' end, primers were selected 3 to the predicted stop codon and used together with primers and reagents in a 5 RACE PCR amplification kit obtained from Clontech to generate a cDNA product coding for PROST 07. A sample of cDNA generated from prostate tissue RNA from Clontech Laboratories was used as a template for this synthesis. These PCR products were cloned into a TA vector obtained from Invitrogen, and selected clones sequenced. This sequence was identical to the nucleotide sequence of *prost 07* predicted by assembly of the set of individual clones in LifeSeq, and contained additional sequence covering the predicted full-length coding sequence of the active form of PROST 07, as well as the putative pro-sequence and part of a putative signal sequence.

Additional confirmatory sequence information was obtained from a genomic clone coding for PROST 07, (P1 clone onc77). Sequence containing the 3' region of the transcript was obtained by sequencing Incyte Clone #1818001 that included the complete 3' region.

A. 5' RACE (5' rapid amplification of cDNA end). 5' RACE-PCR was performed for *prost 07* using the Clontech Marathon cDNA Amplification kit. Normal human prostate mRNA (Clontech) was used as the template. First strand cDNA synthesis was primed using either the Marathon cDNA synthesis primer or a gene-specific primer for *prost 07*. After second-strand synthesis and adaptor ligation, PCR amplifications were performed either with the kit Advantage KlenTaq polymerase mix, AmpliTaq, or AmpliTaq-Gold. Nested PCR reactions were then performed, and PCR products were gel isolated and TA cloned into pCR2.1 using the TA Cloning kit from Invitrogen.

Plasmid DNA was prepared from individual transformant colonies and confirmed by Southern blot using a *prost 07*-specific oligonucleotide probe. Confirmed clones were then sequenced to obtain additional 5 sequence for *prost 07*.

Sequences of PCR primers and oligonucleotide probes:

Prost 07 RACE Set #1

Pr7GSP1

5'-TCCACCATCTGGCTCCCTGGCTC-3' (SEQ ID NO: 3)

Pr7NGSP1

5'-AGGCCCAGCCCGATGGTGTAGGAG-3' (SEQ ID NO: 4)

Pr7Probe1
5'-GCACCAGGACGCCCGAGCAGAACAATTCGTTTTCCATGACCAGTGCCGCC-3' (SEQ ID NO: 7)

Pr7Probe2
5'-TCTGGAAACAGTGTGCGGCTGACAGCACCCACTGCGGATGCACCAGGACG-3' (SEQ ID NO: 8)

Prost 07 RACE Set #2

Pr7GSP2
5'-CTGACAGCACCCACTGCGGATGCAC-3' (SEQ ID NO: 5)

Pr7NGSP2
5'-GAACAATTCGTTTTCCATGACCAGTGCC-3' (SEQ ID NO: 6)

Pr7Probe3
5'-TGCCAGGGCTGCGAGTGCGGGCTGCAGTCC-3' (SEQ ID NO: 9)

B. DNA and protein sequence analysis. The protein sequence predicted by the summed cDNA and genomic sequences was that of an activated form of a serine protease. (Figure 2). Comparison of the nucleotide sequence of *prost 07* with that of known serine proteases including EMSP1 revealed that all the sequence required to code for an active enzyme was present. Additional 5' sequence coded for predicted pro-sequence, and some signal peptide sequence.

Example 2: *Prost 07* mRNA expression

The expression of *prost 07* mRNA in a variety of samples from normal and tumor tissues and in cell lines, was determined by 2 methods: Northern blot analysis and semi-quantitative PCR using a Taqman assay, (Perkin-Elmer). Prostate normal, benign and tumor tissue samples that had been graded according to a modified Gleason grading system were obtained from the Urology Department at Stanford University School of Medicine. RNA was isolated from these by standard procedures. RNA from other tumor and normal tissues was purchased from commercial sources, including Clonetech, and Biochain. Prostate tumor cell lines, (PC-3, LNCaP and DU145), were obtained from American Type Culture Collection and propagated in culture by standard methods using serum containing medium. Xenograft tumors derived from these cell lines were established in nude mice and harvested from the mice approximately 4-6 weeks after implantation. RNA was isolated from the tumors by standard procedures.

A. Northern blot analysis. Northern blots were performed using 2μg of total RNA. (See Figure 4). RNA was loaded on to a I% agarose gel, that was run for 4 hours at 60 volts,

and subsequently transferred to a Hybond membrane. A radiolabelled probe for the *prost 07* mRNA was generated using *prost 07*-containing plasmid, (pINCY containing Incyte clone 3357511), following isolation of the 3357511 insert from the vector. A random primed radiolabelled probe was generated using a Pharmacia Biotech oligo-labelling kit), and used to probe for the *prost 07* mRNA on the blot. An mRNA species that was 1.6Kb in size was detected. This was specifically expressed in the prostate tissues and in the LNCaP prostate cell line but not in any of the other tissues evaluated. Expression was generally higher in the prostate tumor tissues than in the normal tissue from the prostate.

B. Taqman analysis. Taqman based PCR analysis was performed using the primers: GGG AAC TCT TGC CTC GTT TCT (SEQ ID NO: 14) and GAC ACC ACC GAC ACG TTC AC (SEQ ID NO: 15) and the Taqman probe: FAM-TGCTGGCGAACGGCAGAATGC-TAMRA (SEQ ID NO: 16).

These primers and probe were designed using Perkin Elmer's Primer Express software and were synthesized by Synthetic Genetics. PCR reactions were carried out for 30-40 cycles and quantified using prostate RNA to generate a standard curve for relative comparison. This analysis demonstrated that *prost 07* mRNA was detected in the prostate almost exclusively, confirming the result obtained by Northern analysis. (See Figure 5).

Example 3: Histochemical detection of PROST 07

The presence of PROST 07 in a variety of human tissues, including prostate tissue, was examined using histochemical methods. Histochemical studies were conducted using a polyclonal rabbit (Prost 7.4) or a monoclonal mouse (4B8) antibody as the first antibody (see Example 5, below), followed by an alkaline-phosphatase conjugated antibody. As a negative control, the staining was carried out in the absence of the first antibody or by using a non-specific IgG antibody as a first antibody. Both PROST 07 antibodies showed the same staining pattern when used to determine PROST 07 expression in prostate tissues.

Normal human tissues used in the study included 11 organs such as kidney, liver, lung and prostate. The majority of the prostatic glands and ducts were strongly positive for staining (see Figure 8A). The basal cells were negative for staining. A weak granular deposit was seen over areas of the fibromuscular stroma. Seminal vesicle epithelium showed weak cytoplasmic staining in the majority of cells, but a few foci showed moderate to strong staining. Besides a strong to moderate expression of PROST 07 in prostate, a cytoplasmic blush was occasionally seen within cells of other normal tissues, such as liver (hepatocytes), pancreas (islets of Langerhans), or podocytes within the glomerulus (kidney). The majority of cells in normal tissues were negative for PROST 07 expression.

A detailed analysis of PROST 07 expression in prostate tumor tissue was also

performed. (See Figures 8A and 8B). The expression of PROST 07 in prostate carcinoma (n=2), lymph node (n=3), bone and bone marrow metastases (n=6) derived from prostate tumors was determined . All carcinomas used in this study were found to express PROST 07 protein. Within the carcinomas, the majority of tumor cells were positive for PROST 07 expression, although a range of staining, from weak to strong, varied among cells and areas of the tumor. Glands showing changes of prostate intraepithelial neoplam (PIN) showed moderate to strong staining, similar to hyperplastic areas. A weak granular deposit was seen over most of the fibromuscular stroma. Intraluminal secretions were also strongly positive.

All cases of prostatic carcinoma metastatic to lymph node showed positive staining for PROST 07, as all cases of prostatic carcinoma metastatic to bone marrow. Two out of three cases of protate carcinoma metastatic to bone showed expression of PROST 07 protein. The surrounding normal tissue was not stained.

Example 4: Expression, purification and assay for PROST 07 biological activity

I. GST-PROST 07 Construct

A. Expression of GST-PROST 07 (glutathione-S-transferase-PROST 07 fusion protein). To produce correctly folded and hence potentially active PROST 07, a cDNA fragment encoding amino acid residues 11-234, encompassing the deduced mature PROST 07 protease domain, was amplified using the following primers:

M7 5' CGC GGA TCC GAG AAT CTT TAT TTT CAG ATC ATC AAC GGC GAG GAC TGC 3' (SEQ ID NO: 20)

M9 5' CCG GAA TTC TTA ACT GGC CTG GAC GGT TTT C 3' (SEQ ID NO: 21)

The resulting fragment was ligated into a BamHI/EcoRI restricted pAcSecG2T plasmid (Pharmingen). A stock of recombinant baculovirus was produced as it is well known to those of skill. The recombinant fusion protein, GST-PROST 07, was secreted from insect cells (BTI-TN-5B1-4) infected with the recombinant baculovirus and purified from the supernatant by glutathion chromatography.

B. Purification of GST-PROST 07. Insect cell culture supernatants were clarified by centrifugation (4500 rpm for 30 minutes in a Sorvall H-5000 rotor). The resulting supernatant was filtered (0.22 μm filter) and then loaded onto a FPLC column packed with a 5mL bed volume of glutathion sepharose 4B (Pharmacia cat. # 17-0756-01) using a P-1 pump at a rate of 4 ml/min at 4°C. The column was then connected to a Pharmacia FPLC machine and washed with 15 ml of PBS (phosphate buffered saline) at a flow rate of 1.0 ml/minute. Bound GST-PROST 07 fusion protein was eluted off the column with 15 ml of elution buffer (10 mM

reduced glutathione, 50 mM Tris, 100 mM NaCl, pH 8.0) at a rate of 1.0 ml/ minute. Fractions (1 or 2 ml/fraction) were collected and the elution profile was monitored at A_{280}. Eluted fractions were analyzed by PAGE (polyacrylimide gel electrophoresis), protein concentration determined using the Bio-Rad Protein Assay (cat. # 500-006), Western blotting using both anti-GST and/or anti-PROST 07 antibodies, and enzyme activity was monitored by zymogram gel assays.

C. Enzymatic activity of GST-PROST 07.

1. Zymography: To determine whether the fusion protein exhibits enzymatic activity, a sample of the glutathion eluate was subjected to zymography using a 10% gelatin gel (Fig. 7). 5X sample buffer devoid of β-mercaptoethanol, DTT, or any other reducing agent was appropriately mixed with the samples. The samples were loaded directly onto the gelatin zymogram gel (Novex cat. # EC61752) without preheating. The zymogram was run in SDS running buffer following standard PAGE protocol. After the gel was run, it was removed from the plastic plates and soaked in 50 ml renaturing solution (2.5% Triton X-100) for 60 minutes at 21°C. The gel was then incubated in 100 ml of renaturing buffer (50 mM Tris, 40 mM HCl, 200 mM NaCl, 5 mM $CaCl_2$, 0.2% Brij; available from Novex--cat. # LC2671 for 4 hrs. at 37°C without shaking. Following renaturation, the gel was first stained with Coomassie blue for 30 minutes and then destained for 30 minutes in standard PAGE destaining buffer. As depicted in Fig. 7, the GST-PROST 07 fusion protein exhibited an enzymatic activity, which digested gelatin incorporated in the gel. A second band migrating at an apparent molecular weight corresponding to PROST 07 was likewise active in digesting the gelatin matrix. The band resulted from auto-catalytic cleavage at the engineered thrombin site as verified by N-terminal sequencing (data not shown). This indicated that PROST 07 is an active serine protease.

2. Assay using peptide substrates. PROST 07 activity was also measured by following the hydrolysis of synthetic peptide substrates by HPLC analysis. In a 15 μL reaction volume, 1 to 5 μM of either PROST 07 or activated PROST 07 was added to 1 mM of a peptide substrate (SDLVPRMSENLYFQ (SEQ ID NO: 24) or SDLVPRGSENLYFQ (SEQ ID NO: 25)) and incubated at 37° C for various lengths of time. The buffer for this assay is 25 mM HEPES, pH 7.4. After incubation, 5 μL of 10% TFA was added to stop the reaction and then, a 10 μL sample was injected onto an analytical C18 RP-HPLC column (Phenomenex Jupiter 5 μ 300 A 150 x 4.6 mm Part No. 00F-4053-E0). The column was initially equilibrated in 5% Solvent B, where Solvent A is 0.1% TFA in water while Solvent B is 0.1% TFA in acetonitrile. The peptide fragments were eluted using an acetonitrile gradient from 5 to 35% B in 30 minutes at a flowrate of 1 mL/min. The eluent was monitored at 214 nm.

3. Assay of PROST 07 using chromagenic substrates. PROST 07 activity could also be monitored by following the release of a chromophore from a chromogenic subtrate. Using a 96-well plate to a final 200 μL reaction volume, 1 to 5 μM of either Prost 7 or activated Prost 7 was added to 1 mM of a commercial chromagenic thrombin substrate (American

Diagnostica, Greenwich, CT; Cat No. 238L TH). The rate of reaction was monitored using a plate reader set at 405 nm for 30 minutes. The buffer for this assay is 25 mM HEPES, pH 7.4.

II. 6X-HIS PROST 07 Construct

A. Expression of 6X-His PROST 07 (polyhistidine PROST 07 fusion protein). To improve expression and secretion levels of active PROST 07 protease a cDNA encoding amino acid residues 11-234, encompassing the deduced mature PROST 07 protease domain, was amplified using the following primers:

M8 5' CGC GGA TCC ATC ATC AAC GGC GAG GAC TGC (SEQ ID NO: 22)

M29 5' CCG GAA TTC TTA ATG ATG ATG ATG ATG ATG GCT GCT GCC GCC ACT GGC CTG GAC GGT TTT (SEQ ID NO: 23)

The resulting fragment was ligated into a BamHI/EcoRI restricted pAcGP67-B plasmid (Pharmingen). A stock of recombinant baculovirus was produced as it is well known to those of skill. The recombinant fusion protein, 6X-His PROST 07, was secreted from insect cells (BTI-TN-5B1-4) infected with the recombinant baculovirus and purified from the supernatant.

B. Purification of 6X-HIS PROST 07. Using a 10k MWCO tangential flow membrane, ten liters of conditioned media from insect cells expressing 6X-HIS PROST 07 was concentrated to about 200 mL. Using a 14k dialysis tubing, the concentrate was dialyzed against 2 X 4.0L of Buffer A (50 mM Na_2HPO_4, pH 8.0, 300 mM NaCl).

The dialysate was loaded onto a Pharmacia XK 26/20 packed with 20 mL of Qiagen Ni-NTA column at a flowrate of 5 mL/min. After loading, the column was washed with 100 mL of Buffer A followed by 100 mL of 20 mM Imidazole or 8% Buffer B (50 mM Na_2HPO_4, pH 8.0, 300 mM NaCl, 250 mM Imidazole). The enzyme was eluted using an imidazole gradient from 20 mM to 250 mM (8 to 100% Buffer B). Based on SDS-PAGE, western blots and activity assay, fractions containing PROST 07 were pooled.

Using a 14k dialysis tubing, the Ni-NTA pool was dialyzed against 2 X 1.0L of Buffer C (25 mM HEPES, pH 7.4). The dialysate was loaded onto a Pharmacia Mono-Q HR 5/5 column at a flowrate of 1 mL/min. After loading, the column was washed with 10 mL of Buffer C and the enzyme eluted using a salt gradient from 0 to 500 mM NaCl or 0 to 50% Buffer D (25 mM HEPES, pH 7.4, 500 mM NaCl). Based on SDS-PAGE, western blots and activity assays, fractions containing PROST 07 were pooled.

To activate PROST 07, porcine pancreatic elastase (Sigma E-0258) was added to the Mono-Q pool at a ratio of 2:1, i.e. 1 mg of PROST 07 and 0.5 mg of elastase, and incubated at 37° C for 60 minutes. Using the same conditions stated above, the elastase-treated PROST 07 was then loaded onto a Mono-Q column to remove elastase. Elastase flows through this column. Based on SDS-PAGE, western blots and activity assays, fractions

containing elastase-treated PROST 07 were pooled. Protein concentration of PROST 07 and elastase-treated PROST 07 was determined using Pierce BCA Protein Assay Kit (Pierce 23225). For western blots, either rabbit anti-6X-HIS or mouse anti- PROST 07 was used.

Example 5: Antibody Generation

Rabbit polyclonal antisera were raised against four synthetic peptide sequences derived from the PROST 07 polypeptide sequence. These sequences were selected because of their predicted positions at the surface of the protein, in order to generate antisera that are more likely to recognize surface epitopes. Cysteine residues were replaced with aminobutyric acid (Abu) to aid synthesis. The specific amino acid sequences, positions on the PROST 07 polypeptide and designations for the three peptides are listed below.

Designation	Position	Amino Acid Sequence
Pab1	21-36	SQPWQAALMENELF(Abu) (SEQ ID NO: 10)
Pab2	78-91	EASLSVRHPEYNRP (SEQ ID NO: 11)
Pab4	159-172	SKLYDPLYHPSMF(Abu) (SEQ ID NO: 12)
Pab5	221-234	CKFTEWIEKTVQAS (SEQ ID NO: 13)

Peptides were covalently coupled to keyhole limpet hemocyanin (KLH), via an additional amino- terminal cysteine, for use as immunogen. Similarly, a bovine serum albumin (BSA) conjugate was prepared for the analysis of antisera titers via ELISA.

Two animals were immunized with each peptide. Initial immunizations were performed in Freunds complete adjuvant (0.5 mg/animal), followed by periodic boosts at three week intervals with 0.25 mg/animal in Freunds incomplete adjuvant applied intramuscularly. Periodic test bleeds were taken and antibody titers against the specific BSA-peptide conjugate were measured by ELISA and compared with preimmune sera.

Antisera were tested via Western blot for binding to a purified PROST 07 protein expressed as a 6His-PROST 07 fusion protein in *E. coli*. Antisera which recognized the PROST 07 fusion protein were further tested for their ability to recognize the naturally occurring protein in lysates prepared from: LNCAP tumors, LNCAP cells, PC3 tumors and PC3 cells. Binding specificity was determined by antibody binding in the presence and absence of the homologous and heterologous peptides.

Rabbit polyclonal antisera and murine monoclonal antibodies were also raised against the 6His-PROST 07 fusion protein purified from *E. coli*. Immunogen was purified from E. coli cell lysates using a metal chelating affinity resin (Ni-NTA-Sepharose) following extraction in 8 M urea. Ten murine hybridoma clones were obtained which recognized the PROST 07 fusion protein in a denatured state. These monoclonal antibodies were tested for recognition of baculovirus expressed PROST 07 polypeptide using an ELISA assay. Six of these clones were found to recognize native (catalytically active) PROST 07 (monoclonal antibodies 5F4, 4A12, 8D11, 8H5, 2F9, 3F4, and 4B8).

Example 6: Antibody Neutralization of PROST 07 Activity

Murine monoclonal antibodies raised against PROST 07 expressed in *E.Coli* (see Example 5 above) were tested for their ability to neutralize PROST 07 enzymatic activity, using a commercial chromogenic thrombin substrate obtained from American Diagnostica Greenwich, CT (Cat #238L TH). The chromogen p-nitro-anilide was terminally bound through the carboxyl-end to the peptide HDHHTAR-pNA.2AcOH.

Assay for the ability of antibody to neutralize PROST 07 activity was performed in 96 well plates in 50 mM Hepes buffer, pH 7.4 containing 0.1% PEG-6000. Following purification on Protein Sepharose, monoclonal antibodies were incubated overnight with benzamidine Sepharose to remove contaminating proteases. Sepharose beads were removed by brief centrifugation and 1.6 x 10^{-9} moles (in 75µl) of each mab was preincubated with 1.6 x 10-10 moles (in 25 µl) of purified PROST 07 (see Example 4) on ice. After the preincubation, 100 µl of a 1:10 dilution of the chromogenic substrate was added immediately before measuring the changes in absorbance at 405 nm over a 15 minute period at 2 minute intervals using an automated microtiter plate reader. Controls included substrate alone, monoclonal antibody plus substrate, or the reaction mixture plus buffer, normal mouse IgG, or 10 mM benzamidine.

Example 7: Inhibition of tumor cell migration and invasion by anti-PROST 07 antibodies

The ability of the PROST 07 meutralizing monoclonal antibody, 4B8, to inhibit migration and/or invasion of tumor cells *in vitro* was tested using a Boyden chamber (*Pathology Oncology Research*, Vol 4, 1998) PROST 07 positive CAOV-3 cells (ATCC) were incubated with either the PROST 07 specific monoclonal antibody 4B8 or with a non-specific IgG mouse antibody . For the migration assay, cells were seeded onto transwells whose lower side was coated with fibronectin. For the invasion assay, cells were seeded onto transwells whose upper side was coated with matrigel. The PROST 07 neutralizing

monoclonal antibody, 4B8, partially inhibits the ability of CAOV-3 cells to migrate through fibronectin coated wells (see Figure 10A) and partially inhibits the ability of CAOV-3 cells to invade through matrigel coated wells (see Figure 10B), when compared to the effects of the non-specific IgG mouse antibody or to untreated cells.

Example 8: Effect of specific reduction of *prost 07* mRNA on prostate tumor cell growth

The prostate tumor cell lines, PC-3 and LNCaP were selected for evaluation of the role of PROST 07 in cell proliferation using antisense oligonucleotides to reduce mRNA expression in the selected cell lines. The PC-3 cell line expresses extremely low levels of *prost 07* mRNA while the LNCaP cells express significant levels of *prost 07* mRNA. The antisense oligonucleotides used have the sequences CAACGGGUACUUGCAGGGCCUUG (SEQ ID NO: 17), ACCUCUGCAAAUUCACUGAGUGG (SEQ ID NO: 18), and UACUUGCAGGGCCUUGUGUCUUU (SEQ ID NO: 19) and are referred to as 13577, 13581, and 13598, respectively. These reagents are RNA/DNA hybrid molecules, 23 nucleotides in length. The reagents chosen reduce mRNA levels for PROST 07 when administered to cells in the presence of lipids (NC388 lipid mixture provided by Atugen Corp.). These reagents contain oligonucleotide regions which are complementary to the *prost 07* mRNA, with the specific complementing regions for each of these being noted above. Administration of these sequences to LNCaP cells inhibited proliferation of cells in a dose dependent manner in the range 7.5 to 120 nM oligonucleotide when given as a complex with the lipid NC388. In the same dose range, a control oligonucleotide mixture (GBC 3.3) composed of a set of random 23mer oligonucleotides did not affect proliferation of the cells. An antisense oligonucleotide directed against the enzyme inosine monophosphate dehydrogenase, (IMPDH II), an enzyme required for nucleic acid synthesis also caused growth inhibition in LNCaP cells. (See Fig 6).

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All publications and patents mentioned in the above specification are herein incorporated by reference. While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a polynucleotide which is at least 70% identical to a member selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence set forth in Figure 2 (SEQ ID NO: 2);

(b) a polynucleotide encoding a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polynucleotide encoding a polypeptide comprising amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

and

(d) polynucleotide which is complementary to the polynucleotide of (a), (b) or (c).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein the polynucleotide encodes the polypeptide comprising amino acids 1 to 234 as set forth in Figure 2 (SEQ ID NO: 2).

6. The polynucleotide of Claim 2 wherein the polynucleotide encodes the polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2).

7. The polynucleotide of Claim 2 wherein the polynucleotide encodes the polypeptide comprising amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2).

8. The polynucleotide of Claim 1 wherein the polynucleotide comprises the sequence as set forth in Figure 1 (SEQ ID NO: 1) from nucleotide 1 to nucleotide 1354.

9. The polynucleotide of Claim 1 wherein the polynucleotide comprises the sequence as set forth in Figure 1 (SEQ ID NO: 1) from nucleotide 31 to nucleotide 1354.

10. The polynucleotide of Claim 1 wherein the polynucleotide comprises the sequence as set forth in Figure 1 (SEQ ID NO: 1) from nucleotide 10 to nucleotide 1354.

11. A vector comprising the polynucleotide of Claim 2.

12. A host cell comprising the vector of Claim 11.

13. A method of producing a polypeptide comprising expressing from the host cell of Claim 12 the polypeptide encoded by the polynucleotide.

14. The method of Claim 13 wherein the polypeptide comprises amino acid 1 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2).

15. The method of Claim 13 wherein the polypeptide comprises amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2).

16. The method of Claim 13 wherein the polypeptide comprises amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2).

17. A method of producing a polypeptide wherein the polypeptide comprises the amino acid sequence shown in Figure 2 (SEQ ID NO: 2), the method comprising the steps of:

(a) culturing the host cell of Claim 12 under conditions whereby the polypeptide is expressed; and

(b) recovering the polypeptide from the culture.

18. A method for producing a cell which expresses a polypeptide comprising genetically engineering the cell with the vector of Claim 11.

19. A polypeptide comprising a member selected from the group consisting of:

(a) a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2);

(b) a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polypeptide comprising amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(d) a polypeptide comprising amino acid 21 to amino acid 36 as set forth in Figure 2 (SEQ ID NO: 2);

(e) a polypeptide comprising amino acid 78 to amino acid 91 as set forth in

Figure 2 (SEQ ID NO: 2);

(f) a polypeptide comprising amino acid 159 to amino acid 172 as set forth in Figure 2 (SEQ ID NO: 2);

(g) a polypeptide comprising amino acid 221 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2); and

(h) a polypeptide which is at least 70% identical to the polypeptide of (a), (b), (c), (d), (e), (f), or (g).

20. The polypeptide of Claim 19 wherein the polypeptide comprises amino acids 1 to 234 as set forth in Figure 2 (SEQ ID NO: 2).

21. The polypeptide of Claim 19 wherein the polypeptide comprises amino acids 11 to 234 as set forth in Figure 2 (SEQ ID NO: 2).

22. The polypeptide of Claim 19 wherein the polypeptide comprises amino acids 4 to 234 as set forth in Figure 2 (SEQ ID NO: 2).

23. An isolated antibody, or antibody fragment, which specifically binds to a polypeptide comprising a member selected from the group consisting of:

(a) a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2);

(b) a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polypeptide comprising amino acid 21 to amino acid 36 as set forth in Figure 2 (SEQ ID NO: 2);

(d) a polypeptide comprising amino acid 78 to amino acid 91 as set forth in Figure 2 (SEQ ID NO: 2);

(e) a polypeptide comprising amino acid 159 to amino acid 172 as set forth in Figure 2 (SEQ ID NO: 2);

(f) a polypeptide comprising amino acid 221 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2); and

(g) a polypeptide which is at least 70% identical to the polypeptide of (a), (b), (c), (d), (e), or (f).

24. The antibody of Claim 23, wherein the antibody specifically binds to the amino acid sequence SQPWQAALMENELF (SEQ ID NO: 10).

25. The antibody of Claim 23, wherein the antibody specifically binds to the amino acid sequence EASLSVRHPEYNRP (SEQ ID NO: 11).

26. The antibody of Claim 23, wherein the antibody specifically binds to the amino acid sequence SKLYDPLYHPSMF (SEQ ID NO: 12).

27. The antibody of Claim 23, wherein the antibody specifically binds to the amino acid sequence CKFTEWIEKTVQAS (SEQ ID NO: 13).

28. The antibody of Claim 23, wherein the antibody is a polyclonal antibody.

29. The antibody of Claim 23, wherein the antibody is a monoclonal antibody.

30. An immunoconjugate comprising an isolated antibody, or antibody fragment, which specifically binds to a polypeptide comprising a member selected from the group consisting of:

(a) a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2);

(b) a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polypeptide comprising amino acid 21 to amino acid 36 as set forth in Figure 2 (SEQ ID NO: 2);

(d) a polypeptide comprising amino acid 78 to amino acid 91 as set forth in Figure 2 (SEQ ID NO: 2);

(e) a polypeptide comprising amino acid 159 to amino acid 172 as set forth in Figure 2 (SEQ ID NO: 2);

(f) a polypeptide comprising amino acid 221 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2); and

(g) a polypeptide which is at least 70% identical to the polypeptide of (a), (b), (c), (d), (e), or (f) conjugated to a therapeutic agent.

31. The immunoconjugate of Claim 30, wherein the therapeutic agent is a cytotoxic agent.

32. The immunoconjugate of Claim 31, wherein the cytotoxic agent is selected from the group consisting of ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposied, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, *Pseudomonas* exotoxin (PE) A, PE40, ricin, abrin,

glucocorticoid and radioisotopes.

33. The immunoconjugate of Claim 30, wherein the antibody fragments are selected from the group consisting of Fv, F(ab') and $F(ab')_2$ fragments.

34. A method for selectively destroying a cell expressing the polypeptide of Figure 2 (SEQ ID NO: 2) comprising reacting the immunoconjugate of Claim 30 with the cell so that the therapeutic agent of the immunoconjugate can destroy the cell.

35. A method of treating a disease-state in a human patient which disease-state is associated with expression of PROST 07 and wherein the method comprises administering to the patient a therapeutically effective amount of the immunoconjugate of Claim 30.

36. A method of treating a disease-state in a human patient which disease-state is associated with inappropriate expression of PROST 07 and wherein the patient is in need of decreased levels of a polypeptide comprising a member selected from the group consisting of:

(a) a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2);

(b) a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polypeptide comprising amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

and

(d) a polypeptide which is at least 70% identical to the polypeptide of (a), (b), or (c) and wherein the method comprises administering to the patient a therapeutically effective amount of a ribozyme which specifically cleaves RNA encoding the polypeptide.

37. A method of treating a disease-state in a human patient which disease-state is associated with inappropriate expression of PROST 07 and wherein the patient is in need of decreased levels of a polypeptide having the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2); wherein the method comprises administering to the patient a therapeutically effective amount of a polynucleotide which is complementary to a polynucleotide encoding the polypeptide or a portion thereof.

38. The method of Claim 37 wherein the polynucleotide administered has the sequence CAACGGGUACUUGCAGGGCCUUG (SEQ ID NO: 17).

39. The method of Claim 37 wherein the polynucleotide administered has the sequence ACCUCUGCAAAUUCACUGAGUGG (SEQ ID NO: 18).

40. The method of Claim 37 wherein the polynucleotide administered has the sequence UACUUGCAGGGCCUUGUGUCUUU (SEQ ID NO: 19).

41. A diagnostic method wherein the method comprises analyzing a sample derived from a host for the presence of a polypeptide comprising a member selected from the group consisting of:

(a) a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence as set forth in Figure 2 (SEQ ID NO: 2);

(b) a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polypeptide comprising amino acid 21 to amino acid 36 as set forth in Figure 2 (SEQ ID NO: 2);

(d) a polypeptide comprising amino acid 78 to amino acid 91 as set forth in Figure 2 (SEQ ID NO: 2);

(e) a polypeptide comprising amino acid 159 to amino acid 172 as set forth in Figure 2 (SEQ ID NO: 2);

(f) a polypeptide comprising amino acid 221 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2); and

(g) a polypeptide which is at least 70% identical to the polypeptide of (a), (b), (c), (d), (e), or (f).

42. The method of Claim 41, wherein analyzing comprises contacting the sample with the antibody or antibody fragment of Claim 23, which specifically bind to the polypeptide and detecting binding of the antibody to the polypeptide in the sample.

43. A diagnostic method wherein the method comprises analyzing for the presence of a polynucleotide comprising a polynucleotide which is at least 70% identical to a member selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide, or a biologically or immunologically active fragment thereof, comprising the amino acid sequence set forth in Figure 2 (SEQ ID NO: 2);

(b) a polynucleotide encoding a polypeptide comprising amino acid 11 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2);

(c) a polynucleotide encoding a polypeptide comprising amino acid 4 to amino acid 234 as set forth in Figure 2 (SEQ ID NO: 2); and

(d) polynucleotide which is complementary to the polynucleotide of (a), (b) or (c).

44. A method for diagnosing in a subject a metastasis associated with the polypeptide of Figure 2 (SEQ ID NO: 2) comprising:

(a) obtaining from the subject a tissue and/or fluid sample;

(b) contacting the sample with the antibody of Claim 23; and

(c) detecting the binding of the antibody with the polypeptide in the sample.

45. The method of Claim 44, wherein the antibody is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore and a fluorescer.

```
   1  GGATCGCTCG TCTCTGGTAG CTGCAGCCAA ATCATAAACG GCGAGGACTG
  51  CAGCCCGCAC TCGCAGCCCT GGCAGGCGGC ACTGGTCATG GAAAACGAAT
 101  TGTTCTGCTC GGGCGTCCTG GTGCATCCGC AGTGGGTGCT GTCAGCCGCA
 151  CACTGTTTCC AGAACTCCTA CACCATCGGG CTGGGCCTGC ACAGTCTTGA
 201  GGCCGACCAA GAGCCAGGGA GCCAGATGGT GGAGGCCAGC CTCTCCGTAC
 251  GGCACCCAGA GTACAACAGA CCCTTGCTCG CTAACGACCT CATGCTCATC
 301  AAGTTGGACG AATCCGTGTC CGAGTCTGAC ACCATCCGGA GCATCAGCAT
 351  TGCTTCGCAG TGCCCTACCG CGGGGAACTC TTGCCTCGTT TCTGGCTGGG
 401  GTCTGCTGGC GAACGGCAGA ATGCCTACCG TGCTGCAGTG CGTGAACGTG
 451  TCGGTGGTGT CTGAGGAGGT CTGCAGTAAG CTCTATGACC CGCTGTACCA
 501  CCCCAGCATG TTCTGCGCCG GCGGAGGGCA AGACCAGAAG GACTCCTGCA
 551  ACGGTGACTC TGGGGGGCCC CTGATCTGCA ACGGGTACTT GCAGGGCCTT
 601  GTGTCTTTCG GAAAAGCCCC GTGTGGCCAA GTTGGCGTGC CAGGTGTCTA
 651  CACCAACCTC TGCAAATTCA CTGAGTGGAT AGAGAAAACC GTCCAGGCCA
 701  GTTAACTCTG GGGACTGGGA ACCCATGAAA TTGACCCCCA AATACATCCT
 751  GCGGAAGGAA TTCAGGAATA TCTGTTCCCA GCCCCTCCTC CCTCAGGCCC
 801  AGGAGTCCAG GCCCCCAGCC CCTCCTCCCT CAAACCAAGG GTACAGATCC
 851  CCAGCCCCTC CTCCCTCAGA CCCAGGAGTC CAGACCCCCC AGCCCCTCCT
 901  CCCTCAGACC CAGGAGTCCA GCCCCTCCTC CCTCAGACCC AGGAGTCCAG
 951  ACCCCCCAGC CCCTCCTCCC TCAGACCCAG GGGTCCAGCC TCTCCTCCCT
1001  CAGACCCAGG AGTCCAGACC CCCCAGCCCC TCCTCCCTCA GACCCAGGAG
1051  TCCAGCCCCT CCTCCCTCAG ACCCAGGAGT CCAGATCCCC CAGCCCCTCC
1101  TCCCTCAGAC CCAGGGGTCC AGGCCCCCAA CCCCTCCTCC CTCAGACTCA
1151  GAGGTCCAAG CCCCCAACCC CTCCTTCCCC AGACCCAGAG GTCCAGGTAC
1201  CAGCCCCTCC TCCCTCAGAC CCAGCGGTCC AATGCCACCT AGACTCTCCC
1251  TGTACACAGT GCCCCCTTGT GGCACGTTGA CCCAACCTTA CCAGTTGGTT
1301  TTTCATTTTT TGTCCCTTTC CCCTAGATCC AGAAATAAAG TCTAAGAGAA
1351  GCGCAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1401  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1451  AAAAAA
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FIG. 1

```
  1  GSLVSGSCSQ IINGEDCSPH SQPWQAALVM ENELFCSGVL VHPQWVLSAA
 51  HCFQNSYTIG LGLHSLEADQ EPGSQMVEAS LSVRHPEYNR PLLANDLMLI
101  KLDESVSESD TIRSISIASQ CPTAGNSCLV SGWGLLANGR MPTVLQCVNV
151  SVVSEEVCSK LYDPLYHPSM FCAGGGQDQK DSCNGDSGGP LICNGYLQGL
201  VSFGKAPCGQ VGVPGVYTNL CKFTEWIEKT VQAS*
```

FIG. 2

```
  1 MTAAGNPWGWFLGYLLLGITGSLAFINGGHIINGEDCNPHSQPWQAALFL 50
                          |||    .   |||||||.|||||||||||  :
  1 ....................GSLVSGSCSQIINGEDCSPHSQPWQAALVM 30

 51 EDDFFCGGVLVHPQWVLSAAHCFQNSYTIGLGLHNLEPEQEPGSQMMEAS 100
    |.:  || |||||||||||||||||||||||||||.||  :||||||||.|||
 31 ENELFCSGVLVHPQWVLSAAHCFQNSYTIGLGLHSLEADQEPGSQMVEAS 80

101 LSIQHPEYNEPSMANDLMLIKLKESVSLSDTVRNISVVSQCPTPGDSCLV 150
    ||:.||||||  | :||||||||||  ||||  |||:|.||:  |||||  |.||||
 81 LSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLV 130

151 SGWGRLASGRLPQVLQCVNISVASEEVCKARYGPVYHPSMFCAGGGQDQK 200
    ||||  ||.||:|  |||||||:||  |||||    |  |.|||||||||||||||
131 SGWGLLANGRMPTVLQCVNVSVVSEEVCSKLYDPLYHPSMFCAGGGQDQK 180

201 DSCHGDSGGPLICNGSLQGLVSFGQAQCGQPNVPGVYTNLCKFTDWIQTT 250
    |||.||||||||||||  |||||||||.|  |||   ||||||||||||:||:  |
181 DSCNGDSGGPLICNGYLQGLVSFGKAPCGQVGVPGVYTNLCKFTEWIEKT 230

251 IQAS* 255
    :|||||
231 VQAS* 235
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FIG. 3

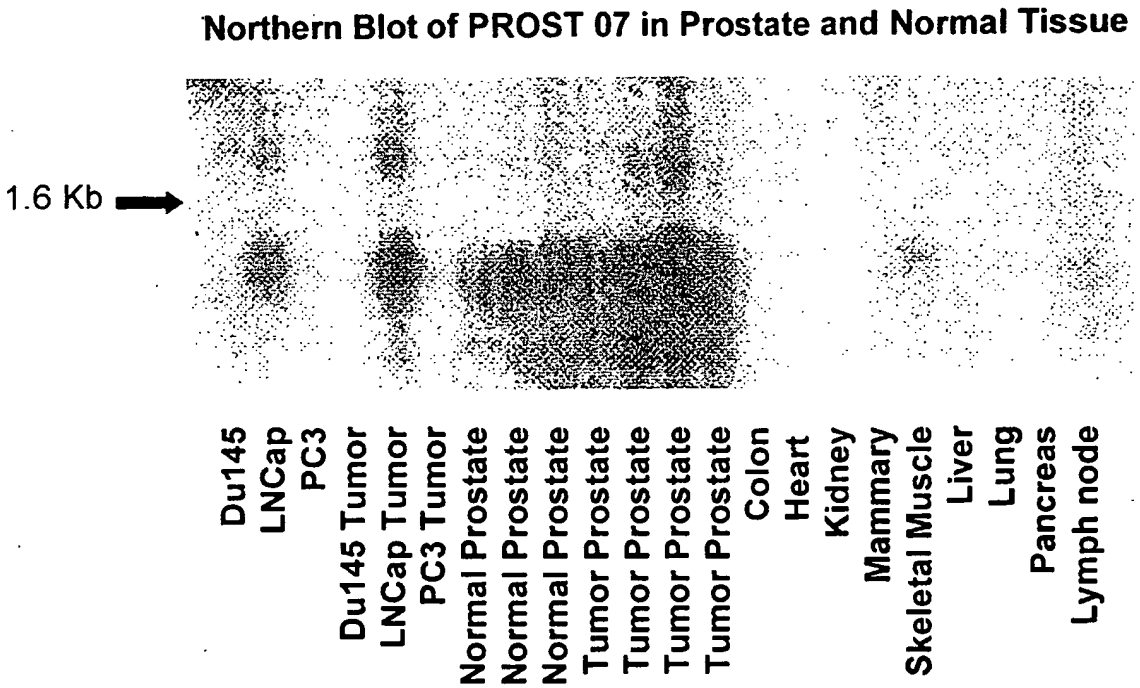

Northern Blot of PROST 07 in Prostate and Normal Tissue
1.6 Kb
Du145
LNCap
PC3
Du145 Tumor
LNCap Tumor
PC3 Tumor
Normal Prostate
Normal Prostate
Normal Prostate
Tumor Prostate
Tumor Prostate
Tumor Prostate
Tumor Prostate
Colon
Heart
Kidney
Mammary
Skeletal Muscle
Liver
Lung
Pancreas
Lymph node

FIG. 4

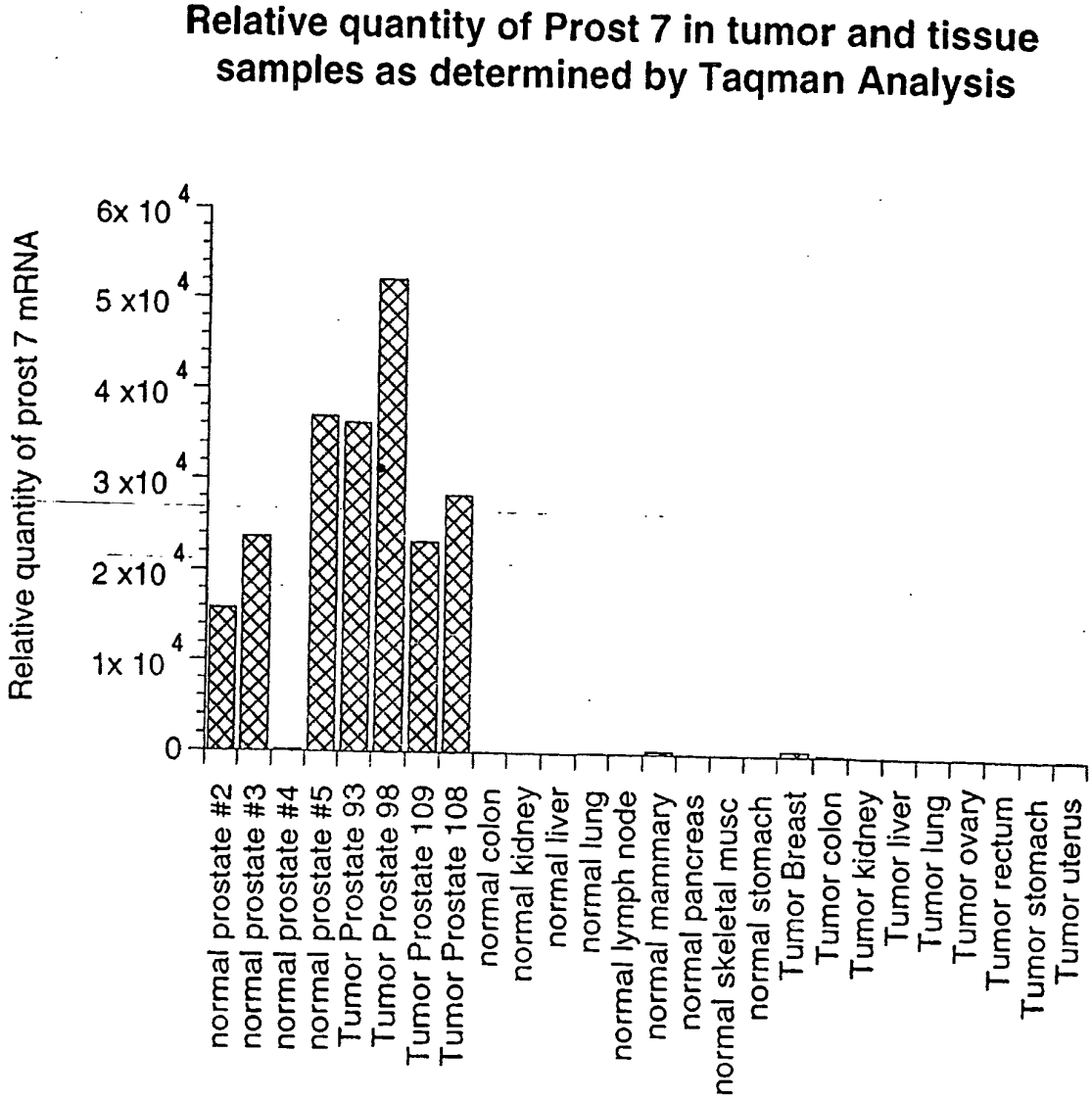

Relative quantity of Prost 7 in tumor and tissue samples as determined by Taqman Analysis
Relative quantity of prost 7 mRNA
6x 10 4
5 x10 4
4 x10 4
3 x10 4
2 x10 4
1x 10 4
0
normal prostate #2
normal prostate #3
normal prostate #4
normal prostate #5
Tumor Prostate 93
Tumor Prostate 98
Tumor Prostate 109
Tumor Prostate 108
normal colon
normal kidney
normal liver
normal lung
normal lymph node
normal mammary
normal pancreas
normal skeletal musc
normal stomach
Tumor Breast
Tumor colon
Tumor kidney
Tumor liver
Tumor lung
Tumor ovary
Tumor rectum
Tumor stomach
Tumor uterus

FIG. 5

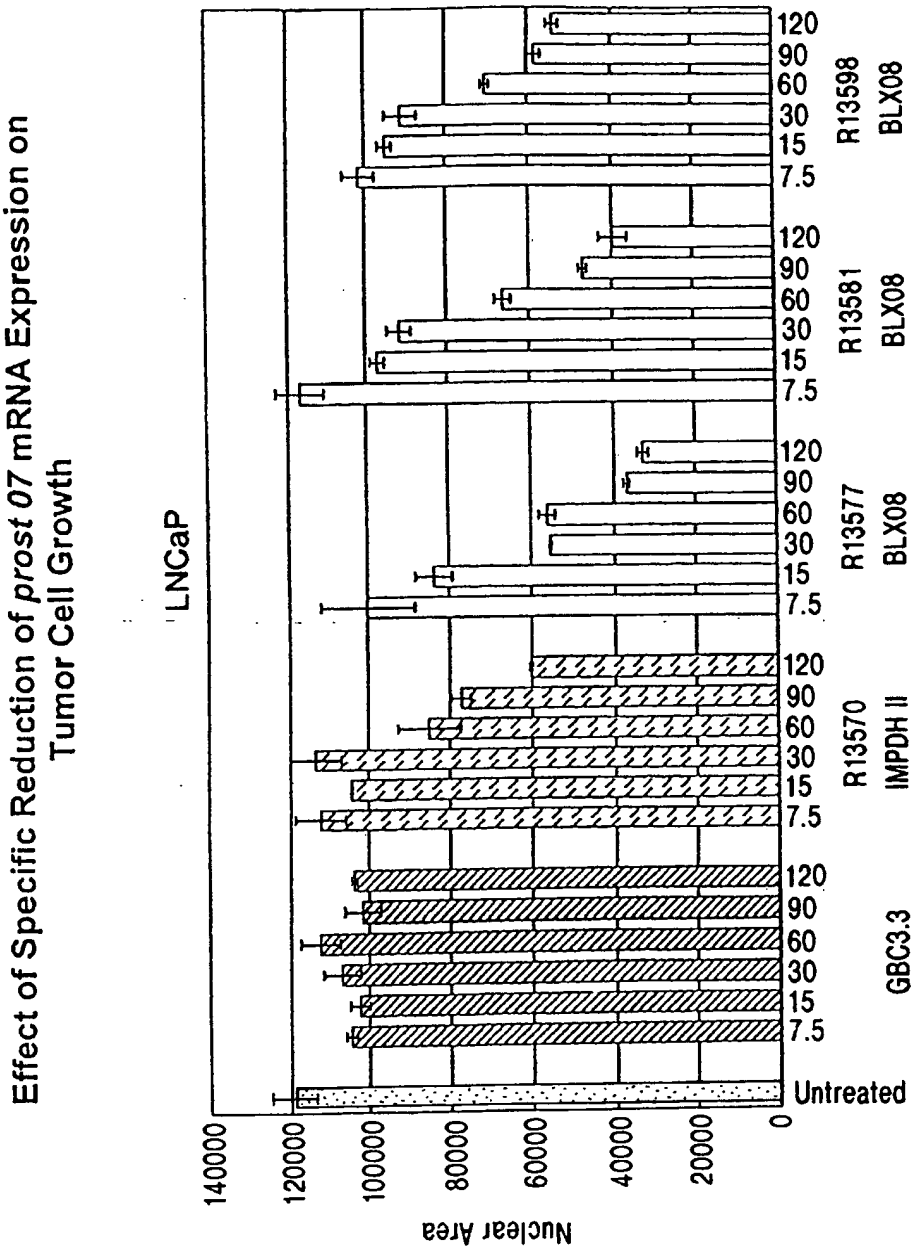

Effect of Specific Reduction of prost 07 mRNA Expression on Tumor Cell Growth
LNCaP
Nuclear Area
140000
120000
100000
80000
60000
40000
20000
0
Untreated
120
90
60
30
15
7.5
R13598
BLX08
R13581
BLX08
R13577
BLX08
R13570
IMPDH II
GBC3.3

FIG. 6

Zymography of purified GST-PROST 07

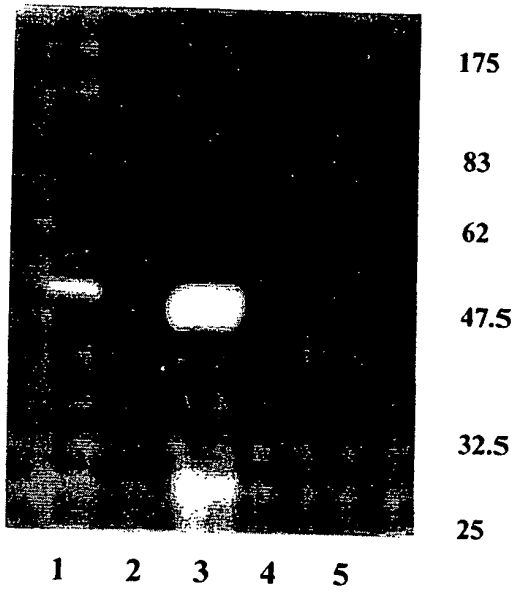


FIG. 7A

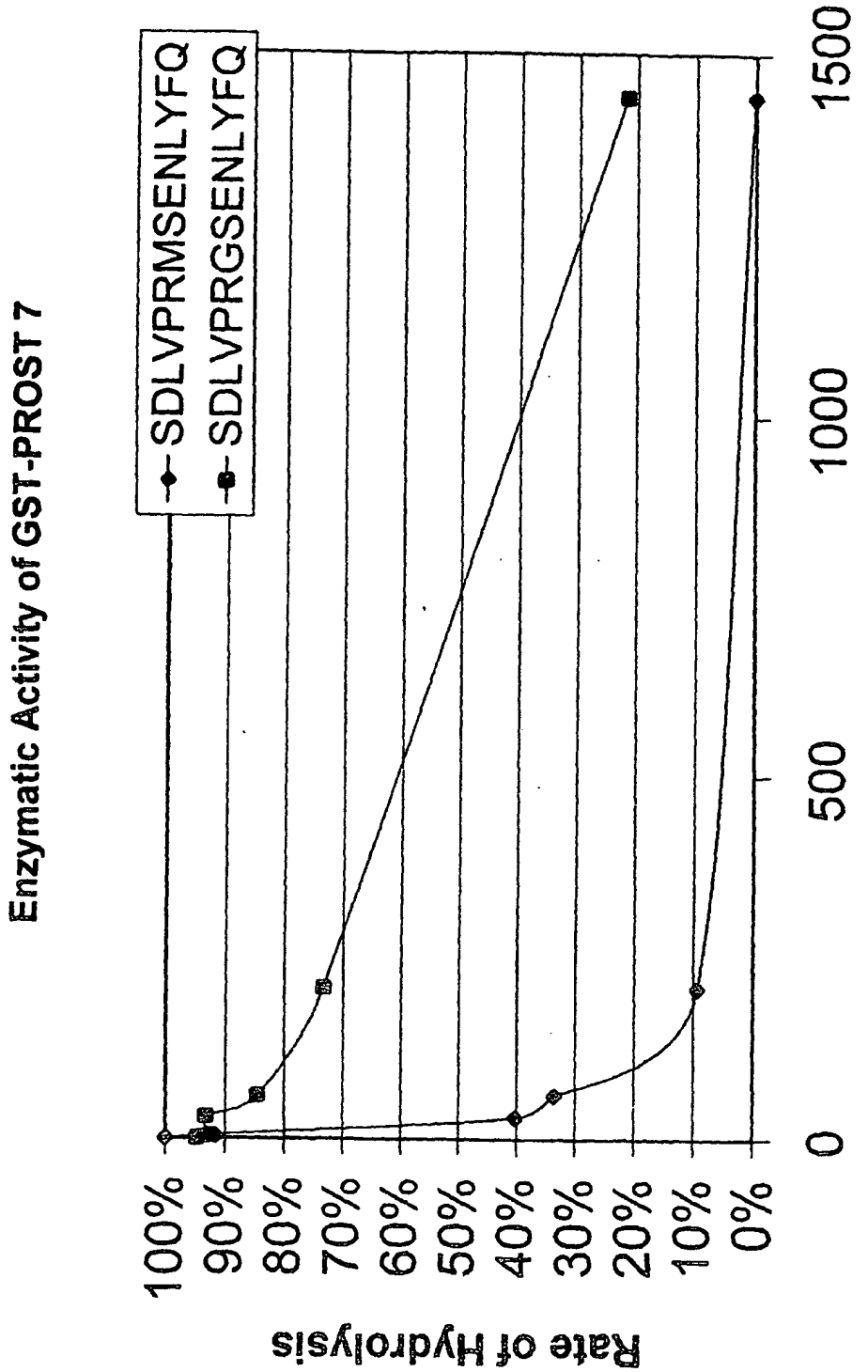

Enzymatic Activity of GST-PROST 7
SDLVPRMSENLYFQ
SDLVPRGSENLYFQ
Rate of Hydrolysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
500
1000
1500
Time (minutes)

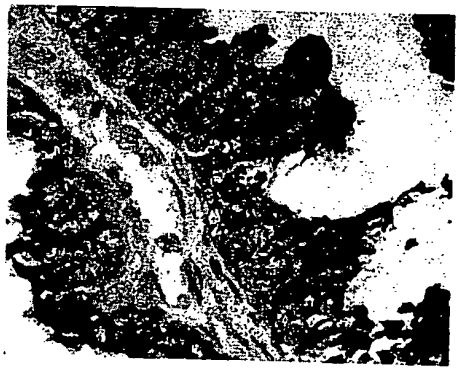
FIG. 7B

Immunohistochemical staining of PROST 07 expression in normal prostate and prostate carcinoma. PROST 07 expressing cells are shown in red.

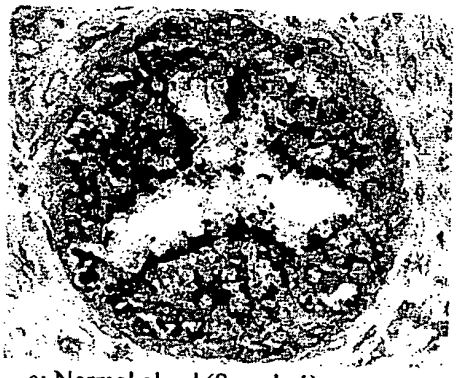
a: Prostate carcinoma (Sample 1)

d: Prostate carcinoma (Sample 2)

b: Prostate carcinoma (Sample 1)



e: Normal gland (Sample 2)

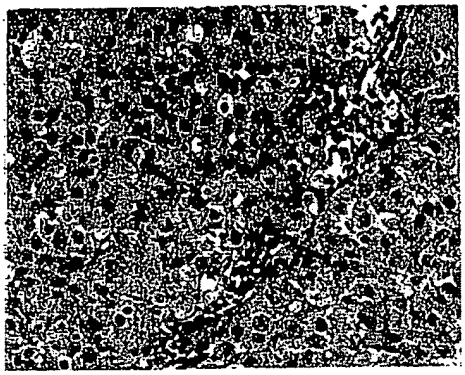


c: Normal gland (Sample 1)

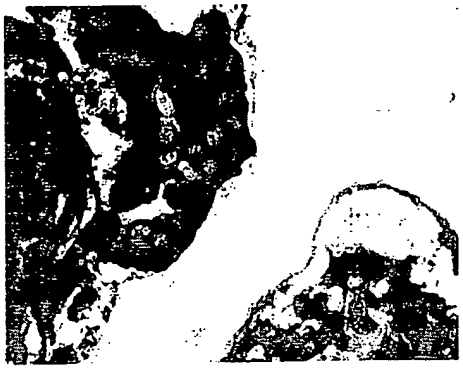
f: Prostatic intraepithelial neoplasia (PIN)

FIG. 8A

Immunohistochemical staining of PROST 07 expression in bone marrow metastasis from prostate cancer. PROST 07 expressing cells are shown in red.

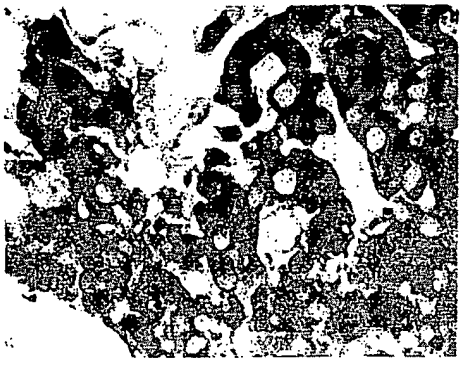


a: Hematoxylin and eosin staining

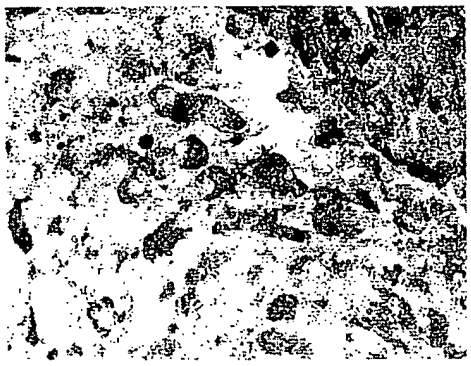


b: Metastatic area in bone marrow (Sample 1)

d: Metastatic area in bone marrow (Sample 3)



c: Metastatic area in bone marrow (Sample 2)



d: Metastatic area in bone marrow (Sample 4)

FIG. 8B

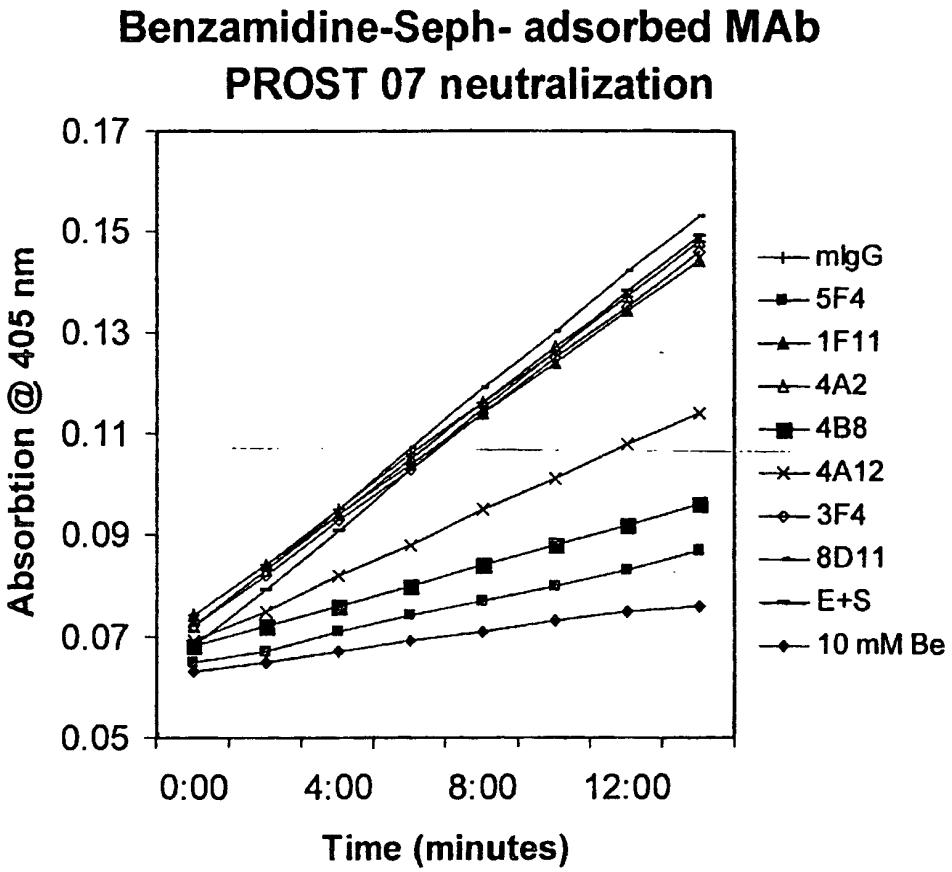
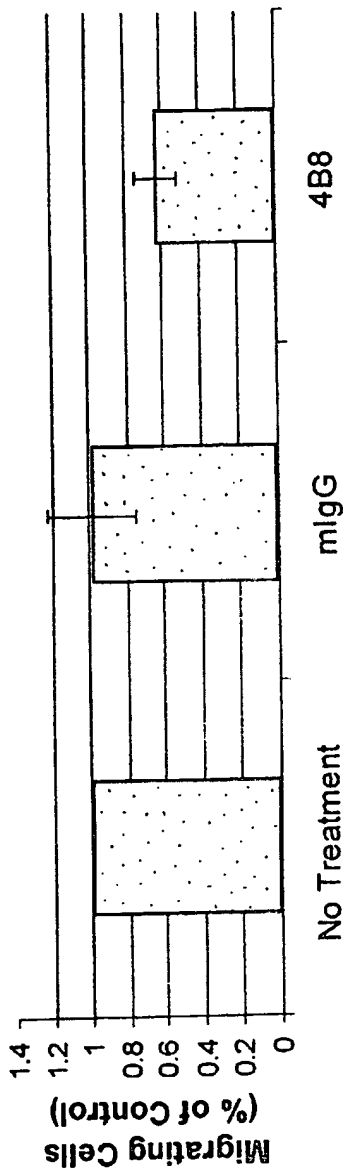
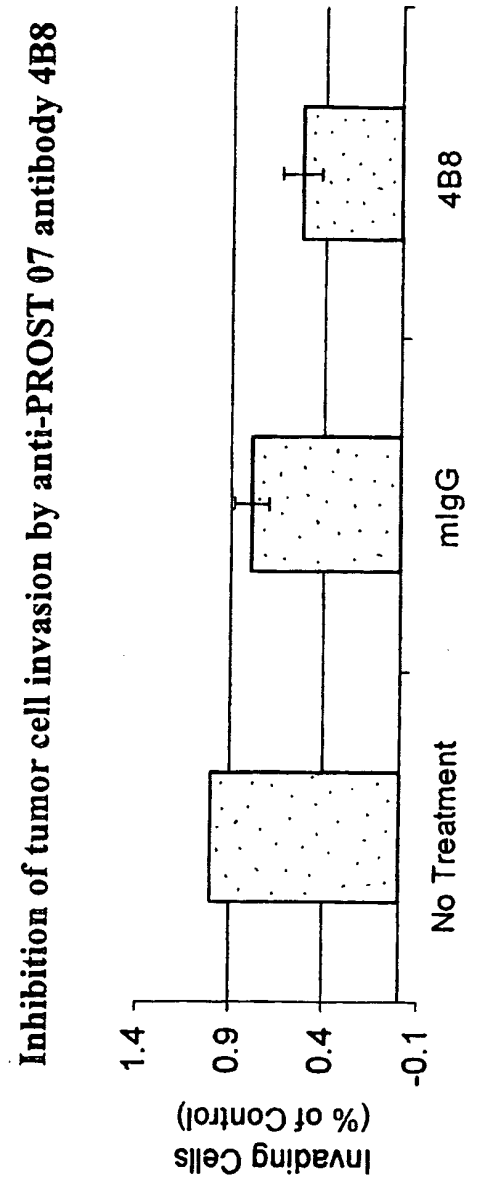

Benzamidine-Seph- adsorbed MAb
PROST 07 neutralization
Absorbtion @ 405 nm
0.17
0.15
0.13
0.11
0.09
0.07
0.05
0:00
4:00
8:00
12:00
Time (minutes)
mIgG
5F4
1F11
4A2
4B8
4A12
3F4
8D11
E+S
10 mM Be

FIG. 9



PROST 07 neutralizing antibody (4B8) can inhibit migration through fibronectin coated transwells using a Boyden chamber. PROST 07 expressing CAOV-3 cells were incubated for 4 hrs with the monoclonal mouse antibody 4B8 (50μg/ml). A non-specific monoclonal mouse antibody (50mg/ml) was used as a negative control. The data were calculated by taking the number of cells migrating in the absence of antibodies (4B8, non-specific antibody) as 100 %. The graph summarizes the results of six independent experiments.

FIG. 10A



PROST 07 neutralizing antibody (4B8) can inhibit invasion through matrigel coated transwells using a Boyden chamber. PROST 07 expressing CAOV-3 cells were incubated for 4 hrs with the monoclonal mouse antibody 4B8 (100μg/ml). A non-specific monoclonal mouse antibody (100mg/ml) was used as a negative control. The data were calculated by taking the number of cells invading in the absence of antibodies (4B8, non-specific antibody) as 100 %. The graph summarizes the results of 4 independent experiments.

FIG. 10B

SEQUENCE LISTING

<110> Bringmann, Peter
Brink, Jody
Harkins, Richard
Light, David
Lin, Richard
Parkes, Deborah
Parry, Gordon
Schneider, Douglas
Steinbrecher, Renate
Toy Van Heuit, Pamela
Xuan, Jian-ai

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tgc agc ccg cac tcg cag ccc tgg cag gcg gca ctg gtc atg gaa aac   96
Cys Ser Pro His Ser Gln Pro Trp Gln Ala Ala Leu Val Met Glu Asn
             20                  25                  30

gaa ttg ttc tgc tcg ggc gtc ctg gtg cat ccg cag tgg gtg ctg tca   144
Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp Val Leu Ser
         35                  40                  45

gcc gca cac tgt ttc cag aac tcc tac acc atc ggg ctg ggc ctg cac   192
Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu Gly Leu His
     50                  55                  60

agt ctt gag gcc gac caa gag cca ggg agc cag atg gtg gag gcc agc   240
Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val Glu Ala Ser
 65                  70                  75                  80

ctc tcc gta cgg cac cca gag tac aac aga ccc ttg ctc gct aac gac   288
Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu Ala Asn Asp
                85                  90                  95
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Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser Asp Thr Ile
            100                 105                 110

cgg agc atc agc att gct tcg cag tgc cct acc gcg ggg aac tct tgc   384
Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly Asn Ser Cys
        115                 120                 125

ctc gtt tct ggc tgg ggt ctg ctg gcg aac ggc aga atg cct acc gtg   432
Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met Pro Thr Val
    130                 135                 140

ctg cag tgc gtg aac gtg tcg gtg gtg tct gag gag gtc tgc agt aag   480
Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val Cys Ser Lys
145                 150                 155                 160

ctc tat gac ccg ctg tac cac ccc agc atg ttc tgc gcc ggc gga ggg   528
Leu Tyr Asp Pro Leu Tyr His Pro Ser Met Phe Cys Ala Gly Gly Gly
                165                 170                 175

caa gac cag aag gac tcc tgc aac ggt gac tct ggg ggg ccc ctg atc   576
Gln Asp Gln Lys Asp Ser Cys Asn Gly Asp Ser Gly Gly Pro Leu Ile
            180                 185                 190

tgc aac ggg tac ttg cag ggc ctt gtg tct ttc gga aaa gcc ccg tgt   624
Cys Asn Gly Tyr Leu Gln Gly Leu Val Ser Phe Gly Lys Ala Pro Cys
        195                 200                 205

ggc caa gtt ggc gtg cca ggt gtc tac acc aac ctc tgc aaa ttc act   672
Gly Gln Val Gly Val Pro Gly Val Tyr Thr Asn Leu Cys Lys Phe Thr
    210                 215                 220

gag tgg ata gag aaa acc gtc cag gcc agt taa ctctggggac tgggaaccca 725
Glu Trp Ile Glu Lys Thr Val Gln Ala Ser
225                 230                 235

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tcctccctca ggcccaggag tccaggcccc cagcccctcc tccctcaaac caagggtaca 845

gatccccagc ccctcctccc tcagacccag gagtccagac cccccagccc ctcctccctc 905

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cctcctccct cagacccagc ggtccaatgc cacctagact ctccctgtac acagtgcccc 1265

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  1               5                  10                  15

Cys Ser Pro His Ser Gln Pro Trp Gln Ala Ala Leu Val Met Glu Asn
             20                  25                  30

Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp Val Leu Ser
         35                  40                  45

Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu Gly Leu His
     50                  55                  60

Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val Glu Ala Ser
 65                  70                  75                  80

Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu Ala Asn Asp
                 85                  90                  95

Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser Asp Thr Ile
            100                 105                 110

Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly Asn Ser Cys
        115                 120                 125

Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met Pro Thr Val
    130                 135                 140

Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val Cys Ser Lys
145                 150                 155                 160

Leu Tyr Asp Pro Leu Tyr His Pro Ser Met Phe Cys Ala Gly Gly Gly
                165                 170                 175

Gln Asp Gln Lys Asp Ser Cys Asn Gly Asp Ser Gly Gly Pro Leu Ile
            180                 185                 190

Cys Asn Gly Tyr Leu Gln Gly Leu Val Ser Phe Gly Lys Ala Pro Cys
        195                 200                 205

Gly Gln Val Gly Val Pro Gly Val Tyr Thr Asn Leu Cys Lys Phe Thr
    210                 215                 220

Glu Trp Ile Glu Lys Thr Val Gln Ala Ser
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1 5 10

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1 5 10

INTERNATIONAL SEARCH REPORT

Interr _ il Application No
PCT/US 00/27431

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N15/11 C12N9/64 C12Q1/68 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 936 270 A (BASF AG) 18 August 1999 (1999-08-18) SEQ ID Nos.1 and 2; claims 1-18 ---	1-23, 28-30, 33,37, 41-45
X	YOUSEF GEORGE M ET AL: "Prostase/KLK-L1 is a new member of the human kallikrein gene family, is expressed in prostate and breast tissues, and is hormonally regulated." CANCER RESEARCH, vol. 59, no. 17, 1 September 1999 (1999-09-01), pages 4252-4256, XP002159639 ISSN: 0008-5472 KLK-L1; Gene bank accession no. AF135023; --- -/--	1-23, 41-45

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2001	21/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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